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(54) **Title**: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

(57) **Abstract**: Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

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SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. provisional patent application number 62/316,555, filed March 31, 2016; U.S. provisional patent application number 62/330,245, filed May 2, 2016; U.S. provisional patent application number 62/372,263, filed August 8, 2016; and U.S. provisional patent application number 62/373,807, filed August 11, 2016, each of which are herein incorporated by reference in their entirety.

SUMMARY

[0002] Provided herein is a brief summary. This summary can be used to provide an understanding of embodiments provided herein, but the embodiments are not limited to what is provided in this summary.

[0003] Provided herein is a pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 6C and at least one additional serotype

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is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 20B and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

[0004] A pharmaceutical composition, which can be in unit dose form, comprising an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof conjugated to a polypeptide, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The pharmaceutical composition can further comprise at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B).

[0005] Provided herein is a method comprising administering to a subject a first composition, wherein the first composition is one of any pharmaceutical composition described herein. Further provided herein is a method comprising administering a first composition and a second composition to a subject, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising an immunogenic saccharide-polypeptide conjugate comprising a second polypeptide to the subject, wherein the second polypeptide

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has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

[0006] Provided herein is a method comprising administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

[0007] Further provided herein is a method of making a composition comprising contacting an immunogenic saccharide-polypeptide conjugate of any one of the described pharmaceutical compositions with an excipient, an adjuvant, or any combination thereof.

[0008] Provided herein is a kit comprising of any pharmaceutical composition described herein or any pharmaceutical composition contained in a container described herein.

[0009] Provided herein are methods of making compositions, methods of immunizing with compositions, and methods of vaccinating with compositions.

INCORPORATION BY REFERENCE

[0010] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0011] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0012] FIGURE 1 illustrates a flow diagram of the general process for manufacture of a saccharide peptide conjugate vaccine.

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[0013] FIGURE 2 illustrates a flow diagram of a process for cell banking of a bacterial strain.

[0014] FIGURE 3 illustrates a flow diagram of a process for producing, isolating, and purifying CRM_{197}.

[0015] FIGURE 4 illustrates a flow diagram of a fermentation process for isolating a polysaccharide from a strain of *Streptococcus pneumoniae*.

[0016] FIGURE 5 illustrates a flow diagram of a conjugation process for conjugating a polysaccharide and CRM_{197}.

[0017] FIGURE 6 illustrates a flow diagram for a process for formulating a polysaccharide polypeptide conjugate vaccine.

[0018] FIGURE 7 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B (ePCV; LiffeyVax Serotypes) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13). Prevnar 13 alone only covers about 46.1% of IPD compared to an overall coverage of 90% when administered in a vaccination regime with ePCV.

[0019] FIGURE 8 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B (Liffey/Vax ePCV8) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of the eight serotypes in the Liffey/Vax ePCV8 is predicted to cover about 23% more of IPD when administered in a vaccination regime with PCV20.

[0020] FIGURE 9 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38 (ePCV8+7) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven

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serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of seven serotypes in the Liffey/Vax ePCV8 (Liffey/Vax ePCV8+7) (see **FIGURE 8** for ePCV8) is predicted to cover about 29% more of IPD when administered in a vaccination regime with PCV20.

[0021] FIGURES 10A, 10B, 10C, 10D, 10E, and 10F illustrate annotated the capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley et al., Genetic Anaylsis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

DETAILED DESCRIPTION

[0022] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognizes that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0023] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0024] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range covering plus or minus up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" can mean within an acceptable error range for the particular value should be assumed.

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[0025] A "unit dose" when used in reference to a pharmaceutical composition can refer to units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect. In some instance the unit dose can contain a diluent; *i.e.*, carrier, or vehicle. In some instances, the unit dose can be a physically discrete.

[0026] The term "saccharide" can refer to a saccharide, oligosaccharide or polysaccharide. For example, saccharide can mean a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexasaccharide, a heptasaccharide, an octasaccharide, a nonasaccharide, or a decasaccharide. An oligosaccharide can refer to two to ten saccharides. A polysaccharide can refer to more than ten saccharides.

[0027] The term "polypeptide" can refer to a single linear chain of amino acids held together by amide bonds. A polypeptide can refer to at least two amino acids. A polypeptide can be an oligopeptide, wherein the term "oligopeptide" can refer to a single chain of from two to twenty amino acids joined by amide bonds. A polypeptide can be a protein, wherein the term "protein" can refer to a single chain of fifty or more amino acids held together by amide bonds.

[0028] The term an "immune response" can refer to the production of cytokines and/or antibodies with a degree of specificity for an antigen after administering the antigen to a subject. In some instances, the term "immune response" can refer to any change in immune cells or change caused by immune cells after administering the antigen to a subject. An immune response can be T cell independent, T cell dependent, or both.

[0029] The term "homology" can mean percent sequence identity between a particular nucleic acid or amino acid sequence and another nucleic acid or amino acid sequence. First, a nucleic acid or amino acid sequence can be compared another sequence using the BLAST 2 Sequences (Bl2seq) program from the stand- alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. BLASTN can be used to compare nucleic acid sequences, while BLASTP can be used to compare amino acid sequences. Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity or homology can be determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (*e.g.*, 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100%.

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[0030] The term "length homology" can mean percent length identity between the length of a particular polypeptide and the length of another polypeptide. "Length homology" can in some instances be calculated by dividing the number of amino acids in a first peptide chain by the number of amino acids in a second peptide chain and multiplying the result by 100%. Chain amino acids can be those forming the backbone of the peptide.

[0031] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0032] The term "immunogenic fragment" can be a fragment that is conjugated to a polypeptide, administered to a subject, and which elicits an immune response. In some instances, the fragment can be a fragment of a capsular polysaccharide. In other instances, the polypeptide can be CRM_{197}.

[0033] Immunogenic, which can but need not be obligated to be preceded any term herein, can mean that which, when administered to a subject, which can be a human or an animal, for example a dog, cat, rat, mouse, sheep, or monkey, produces an immune response in the animal. For example, a dog can be *Canis lupis familiarius*, a cat can be *Felis catus*, a rat can be *Rattus norvegicus*, a mouse can be *Mus musculus*, a sheep can be *Ovis aries*, or a monkey can be *Simia inuus*. The administration can be, for example, subcutaneous, intramuscular, or rectal, for example in the form of a suppository.

[0034] In some embodiments, a unique immunogenic saccharide-polypeptide conjugate can be one that differs from other unique immunogenic saccharide-polypeptide conjugates, for example in a plurality of these, by at least one aspect. For example, the unique immunogenic saccharide-polypeptide conjugate can differ by the saccharide or fragment thereof it contains - in that this can render the unique immunogenic saccharide-polypeptide conjugate unique, or by the polypeptide it contains, or both. In some instances, unique immunogenic saccharide-polypeptide conjugates, to be unique, simply need not be 100% identical.

[0035] The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide.

[0036] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0037] The term "plurality" can mean two or more.

[0038] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

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[0039] Bacteria can incorporate saccharides into their surface structure. For example, *Streptococcus pneumoniae* (*S. pneumoniae*) can contain saccharides in its surface structure. The saccharides can be antigens that can induce an immune response in host, such as an animal or human. For example, B cells can produce antibodies against a saccharide. This response can be a T cell independent immune response. An immune response can be induced with full-length, native form polysaccharides, oligosaccharides, or fragments thereof. An immune response can be induced with full-length, native form capsular polysaccharides, or fragments thereof. Other bacteria that can contain saccharides are *Neisseria meningitidis (N. meningitidis)*, *Salmonella enterica (S. enterica)*, and *Haemophilus influenzae (H. influenzae)*.

[0040] The saccharides on bacteria can be used to classify the bacteria into a subclass or serotype. More specifically, a serotype can refer to the type of a microorganism determined by its constituent antigens. The constituent antigens can be different saccharides, such as different capsular polysaccharides or immunogenic fragments thereof. Some non-limiting examples of *S. pneumoniae* serotypes can include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B).

[0041] By conjugating a saccharide to a polypeptide, the immune response to the saccharide can be enhanced by inducing a T cell dependent response. A saccharide alone may not stimulate T cells because, for example, a saccharide may not be loaded onto the Major Histocompatibility Complex (MHC) of antigen presenting cells. However, peptides can be loaded onto MHC. Therefore, by conjugating a saccharide to a polypeptide, the saccharide-polypeptide conjugate can then be loaded onto the MHC, recognized by the T cell, and stimulate a T cell response. T cells can then stimulate a more vigorous immune response and also promote a more rapid and long-lasting immunologic memory. Provided herein are saccharide-polypeptide conjugates that can be used to induce an immune response, which can be referred to as immunogenic saccharide-polypeptide conjugates. In some instances, the immunogenic saccharide-polypeptide conjugates can provide immune protection against a saccharide or organism containing the saccharides. In other instances, the immunogenic saccharide-polypeptide conjugates can be part of a vaccine.

Compositions of Saccharide-Polypeptide Conjugate

[0042] An immunogenic saccharide-polypeptide conjugate composition can be a saccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition

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can be a capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide or fragment of the capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise a saccharide antigen coupled to one or more polypeptides. An immunogenic saccharide-polypeptide conjugate can comprise one unique saccharide antigen coupled a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise two or more unique saccharide antigens coupled a polypeptide. A saccharide antigen can elicit an immune response.

[0043] A saccharide can be from any bacteria and fungi that can incorporate saccharides into their surface structure. For example, saccharides can be from *S. pneumoniae*. Further examples include saccharides from *N. meningitidis*, *S. enterica*, or *H. influenzae*. A saccharide can be a full-length, native form polysaccharide, oligosaccharide, or fragments thereof. A saccharide can be in any form that induces an immune response. A saccharide can be a capsular polysaccharide or fragment thereof. A saccharide can be from a subclass or serotype. Some non-limiting examples of saccharides from a *S. pneumoniae* serotype include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20. Serogroup 20 can comprise serotype 20A and serotype 20B. *S. pneumoniae* that can produce serotype 20A polysaccharide can be identified by a mutated *whaF* allele as compared to the intact *whaF* allele of *S. pneumoniae* that can produce serotype 20B polysaccharide. **TABLE 1** depicts exemplary repeat unit polysaccharide structures of various *S. pneumonaie* serotypes. **FIGURES 11A, 11B, 11C, 11D**, **11E**, and **11F** depict annotated the capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley et al. Genetic Analysis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

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TABLE 1. Exemplary *S. pneumoniae* Polysaccharide Serotypes

Serotype	Polysaccharide Structure (Repeat Unit)
1	→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→]$_n$ Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→]$_n$ 2 ↓ 1 α-D-Glc*p*A-(1→6)-α-D-Glc*p*
3	→3)-β-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
4	→3)-β-D-Man*p*NAc-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→4)-α-Gal*p*2,3(S)Pyr-(1→]$_n$
5	→4)-β-D-Glc*p*-(1→4)- α -L-Fuc*p*NAc-(1→3)-β-D-Sug*p*-(1→]$_n$ 3 ↑ 1 α-L-Pne*p*NAc-(1-2)-β-D-Glc*p*A L-PneNAc: 2-acetamido-2,6-dideoxy-l-talose (pneumosamine) Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
6A	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitool-(5→P→]$_n$
6B	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Ribitol-(5→P→]$_n$
6C	→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitol-(5→P→]$_n$

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Serotype	Polysaccharide Structure (Repeat Unit)
7F	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 2 4 ↑ ↑ 1 1 β-D-Gal*p* α-D-Gal*p*NAc-(1→2)-α-L-Rha*p*
8	→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→4)-α-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
9N	→4)-α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→]$_n$
9V	→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→]$_n$ ↑ ↑ ↑ 2Ac (17%) 4Ac (6%) 2Ac (3%) 3Ac (25%) 6Ac (55%) 3Ac (4%)
10A	Galβ1 ↓ 6 →4)-GalNAc-β-(1→3)-Gal-α-(1→2)-ribitol-5-PO4$^-$-5Gal*f*β-(1→3)-Galβ-(1→]$_n$ 3 ↑ Gal*f*β1
11A	$OAc_{0.5}$ OAc ↓ ↓ 3 2 →3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→]$_n$ 4 ↑ PO_4-1-glycerol

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Serotype	Polysaccharide Structure (Repeat Unit)
	0.5 OAc undeterminate
12F	→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Man*p*NAc-(1→] 3 3 ↑ ↑ 1 1 α-D-Gal*p* α-D-Glc*p*-(1→2)-α-D-Glc*p*
14	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 β-D-Gal*p*
15A	→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-α-Gal*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
15B	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α -D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline Note: On average 70% of the repeating units is O acetylated. The site of the O acetylation is unknown (Jansson *et al.* Carbohydr. Res. (1987) 162:111-116), although Venkateswaran *et al.* Infect. Dis. (1983) 147:1041-1054 suggested that it is linked to a galactose moiety.

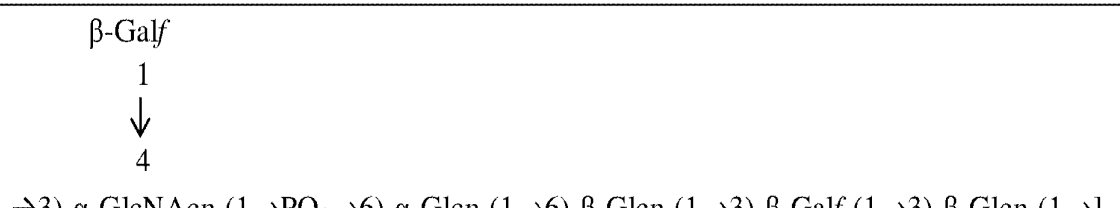
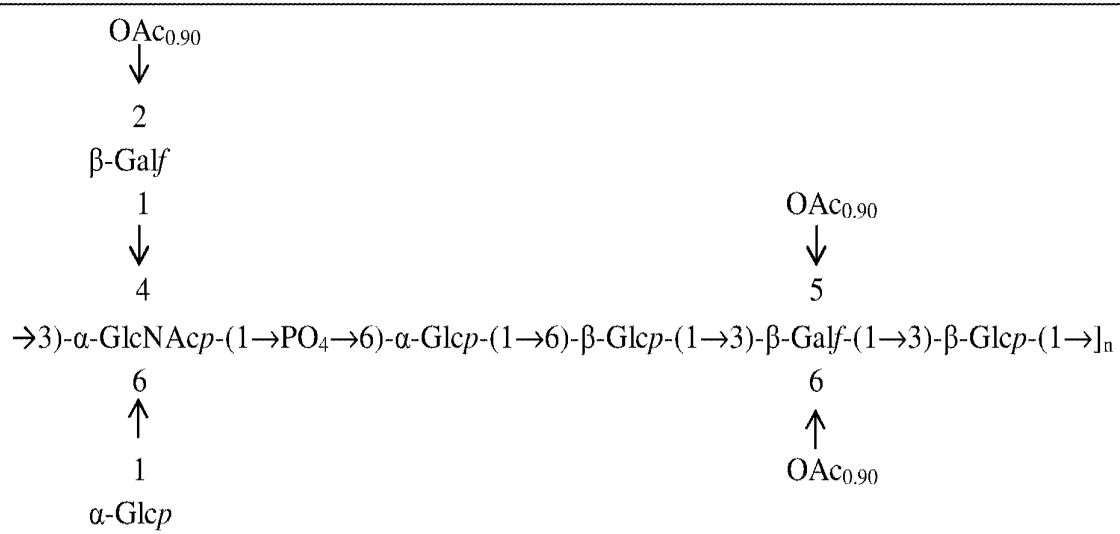
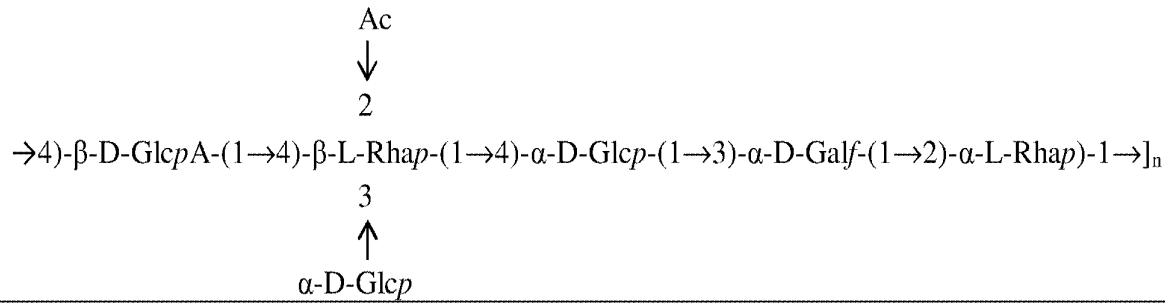
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 4 ↑ 4 β-D-Gal*p* and OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 1 ↑ 4 β-D-Gal*p*

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Serotype	Polysaccharide Structure (Repeat Unit)
18C	α-D-Glc*p* 1 ↓ 2 -[4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rhap-(1→]n 3 ↑ PO4-1-glycerol
19F	→2)-α-L-Rha-(1→5-PO_4^--5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$
19A	→3)-α-L-Rha-(1→5-PO_4^--5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$

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Serotype	Polysaccharide Structure (Repeat Unit)
20A	
20B	
22F	

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Serotype	Polysaccharide Structure (Repeat Unit)
23F	a-L-Rha*p* 1 ↓ 2 →3)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
31	→2)-β-L-Rha*p*-(1→3)-β-D-Gal*f*-(1→3)-β-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→]$_n$
33F	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)– β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*-(1→]$_n$ 2 2 ↑ ↑ 1 $OAc_{0.4}$ α-D-Gal*p*
35F	→6)-Gal*f*-β-(1→3)-Gal-α-(1→2)-Rib-5-PO_4^--3-Gal*f*-β-(1→3)-Gal-β-1→]$_n$ ↓ 2OAc
35B	$OAc_{0.7}$ ↓ 2 →4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*-(1→1)-Rib-(5-PO_4→]$_n$

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[0044] Saccharides can be isolated from bacteria. Saccharides can be isolated from bacteria and then conjugated to a polypeptide to form a saccharide-polypeptide conjugate. Any method for isolating the unconjugated polysaccharide can be used. For example, a polysaccharide serotype can be grown in a soy-based medium. The individual polysaccharides can then be purified by steps including centrifugation, precipitation, and ultra-filtration. *See* U.S. Patent Application Publication No. 2008/028638 and U.S. Pat. No. 5,847,112. Other processes for purifying polysaccharides are described in U.S. Pat. No. 4,686,102; 4,242,501; 4221,906; 5623,057; and 5,847,112. Alternatively or additionally, polysaccharides may be isolated simultaneously from more than one serotype, which can produce a mixture of polysaccharide serotypes with the desired polysaccharide already combined. Furthermore, these isolated polysaccharides can be sized by microfluidization. The polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated sample. A saccharide-polypeptide conjugate can include a saccharide or a fragment thereof which is at least partially embedded in one or more polypeptides. The one or more polypeptides can have one or more crosslinks. The at least partially embedded saccharide or fragment thereof can, but need not be, covalently bound to at least one of the polypeptides when at least partially embedded therein.

[0045] Any of the *S. pneumoniae* strains for isolating and purifying the polysaccharides as described herein can be provided by either the CDC, United States or CCUG, Gothenburg. Alternatively, any of the polysaccharides described herein can be isolated and purified from any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the unique strain from which the polysaccharides are isolated and purified. A vial of a strain of *S. pneumoniae* can be seeded and then can be grown overnight on an agar plate. A single colony can be picked from this plate and streaked onto a new agar plate for overnight growth. This process can be repeated three times to purify the strain before a flask can be seeded and incubated. After allowing the seeded flask sufficient time for growth, the flask contents can be harvested by centrifugation. The centrifugation product can be resuspended in a medium of about 20% glycerol and then can be dispensed into cryogenic vials. The cell bank can be contained in the cryogenic vials, which can be stored at -70°C until use. The cell bank of the strain can then be used for isolation and purification of the polysaccharides of that strain using a fermentation process.

[0046] Polysaccharides of a *S. pneumoniae* strain can be isolated and purified from a cell bank of a strain. The strain can be provided by either the CDC, United States or CCUG, Gothenburg, and then can be used to produce a cell bank as described above. Alternatively,

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strain can be provided by any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the serotype of the strain. A vial of a cell bank can be produced as described above is used to inoculate a flask, which is allowed to grow overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 μm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating). Any polysaccharides described herein can be isolated and purified using this method.

[0047] The saccharides or polysaccharides of the invention are depolymerized (sized) to a final range of 100-500 kDa.

[0048] Saccharides that can be used in the saccharide-polypeptide conjugate can also be obtained from commercial sources. For example, individual polysaccharide serotypes can be obtained in a powder form. These polysaccharides can be dissolved in water and incubated with a salt to dissociate residual impurities, which can then be removed by a filtration step. The purified polysaccharides serotypes can then be conjugated to a polypeptide.

[0049] A polypeptide of an immunogenic saccharide-polypeptide conjugate can be an oligopeptide. A polypeptide of can be a carrier protein. A polypeptide can be any polypeptide that allows for conjugation or coupling of saccharide and results in the display of the conjugate or coupling in a way that induces a protective immune response against the saccharide. A polypeptide can be any polypeptide that allows for conjugation or coupling of a capsular polysaccharide or fragment of the capsular polysaccharide and can result in the display of the conjugate or coupling in a way that can induce a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide. A polypeptide can be CRM_{197}, tetanus toxoid, a diphtheria toxoid, a cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, a keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, or any fragment thereof. The polypeptide can be a polypeptide in which the

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toxin activity of the polypeptide can be at least partially mitigated. Examples of suitable polypeptides for use as in a saccharide-polypeptide conjugate are described in U.S. patent 8,808,707 and can include: polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, *e.g.*, serines, threonines, or tyrosines, to confer desirable solubility properties on the resultant loaded carrier and conjugate. A polypeptide can be a CRM_{197} with 100% sequence homology with GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). A polypeptide can be a CRM_{197} with at least 80%, 90%, 95%, or 98% sequence homology with SEQ ID NO: 1. A polypeptide can be a modified CRM_{197} as disclosed in US Patent Application No. 14/329,758. A polypeptide can be CRM_{197} with non-natural amino acid substitutions as disclosed in US Patent Application No. 14/328,532.

[0050] Moreover, suitable polypeptide carriers can include bacterial toxins, toxoids, or inactivated toxin. A toxoid can be a bacterial toxin whose toxicity has been weakened or suppressed while other properties, typically immunogenicity, are maintained. As a class, bacterial toxins and derivatives thereof can to be highly immunogenic. Polypeptides derived from bacterial toxins can be effective at eliciting an immune response to saccharides of the conjugate. Steps can be taken (*e.g.*, by chemical and/or genetic means) to render the toxins non-toxic and safe for administration to mammals. Examples of such bacterial toxin-derived polypeptides which may currently commonly used in vaccine compositions, and can be used in the immunogenic saccharide-polypeptide conjugate described herein, include the diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from

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N. meningitidis, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* PspA, or any fragment thereof. Polypeptide fragments can be used to elicit a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide of the immunogenic saccharide-polypeptide conjugate. These fragments can encompass T-helper epitopes.

[0051] CRM_{197} is a non-toxic mutant of diphtheria toxin with a single amino acid substitution of glutamic acid for glycine at position 52, with the amino acid sequence of GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). CRM_{197} can be from 58-59 kDa in size. CRM_{197} can be 58.4 kDa in size. CRM_{197} can be produced, isolated, and purified from a cell bank. A vial from a cell bank of CRM_{197} can be used to seed a new flask and allowed to ferment. The fermentation culture can be centrifuged and the contents can be harvested, clarified, and filtered using a 0.2 μm filter. The filtrate can then be concentrated and diafiltered. The CRM_{197} can be further purified by filtration using microtubes or membranes of the appropriate pore size. Then anion exchange chromatography can be used in the isolation and purification of the CRM_{197}, which can be followed by ammonium sulphate precipitation, and another filtration using a 0.45 μm filter. CRM_{197} can be further isolated and purified from this product by hydrophobic interaction chromatography, after which CRM_{197} can be concentrated and diafiltered into the desired buffer. A final step can be filtration with 0.2 μm filter. The isolated and purified CRM_{197} can be stored at ≤ -70°C until use in conjugation with a saccharide or polysaccharide. Quality control tests can performed on the isolated and purified CRM_{197}.

[0052] A polypeptide can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 2 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be

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conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 17F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A

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polypeptide can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.*

[0053] In some embodiments, CRM_{197} can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* CRM_{197} can be conjugated to an immunogenic serotype 2 capsular

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polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 17F capsular polysaccharide,

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fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*.

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[0054] In further embodiments, any of the saccharides as described herein can be conjugated to any polypeptides described herein. Any of the immunogenic saccharide-polypeptide conjugates described herein can be isolated and purified.

Methods of Conjugating a Saccharide and Polypeptide to Produce a Saccharide-Polypeptide Conjugate

[0055] The saccharides can be conjugated to the polypeptide by, *e.g.*, chemical or biological synthesis. A saccharide can be a polysaccharide, a capsular polysaccharide, or an immunogenic fragment thereof. A capsular polysaccharide or fragment of the capsular polysaccharide can be conjugated to the polypeptide by chemical or biological synthesis.

[0056] For example, the capsular polysaccharide or immunogenic fragment thereof can be chemically activated and then reacted with the polypeptide. For example, capsular polysaccharides or fragments of the capsular polysaccharides can be reacted with either ammonia or diaminoethane to generate a free terminal amino group. The amino group can then be reacted with an excess of disuccinimidyl ester of adipic acid, which can introduce an active succininmidyl ester group. This can then be reacted to a free amino group on the polypeptide to form covalent amide bonds.

[0057] A polysaccharide and polypeptide can be conjugated using 1-cyano-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry. CDAP can be used to activate a broad range of acidic, neutral, and other classes of polysaccharides, which can then be derivatized with diamines, dihydrazides or other functional groups in preparation for coupling to proteins. In CDAP, the cynanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate can be used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which can avoid hydrolysis of the alkaline sensitive polysaccharides. This synthesis can allow for direct coupling to a carrier. The polysaccharide can be solubilized in water or a saline solution. CDAP can be dissolved in acetonitrile and can be added immediately to the polysaccharide solution. The CDAP can reacts with the hydroxyl groups of the polysaccharide to form a cyanate ester. This cyanate ester can be highly reactive, which can result in an activated polysaccharide. This can be done at a pH of 9–10. After this activation step, the polypeptide can be added. Amino groups of lysine of a polypeptide can react with the activated polysaccharide to form an isourea covalent link. This conjugation step can occur under mildly alkaline conditions, such as a pH of 7–9. In some cases, there can be a strong pH dependence on the epsilon amines of lysine to form a stable O-alkyl-isourea linkage. After the coupling

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reaction, a large excess of an amino-containing reagent such as glycine can then be added to quench the residual activated functional groups. The CDAP activation can take only a few minutes and conjugation can be completed in a few hours. The conjugate can then be passed through a gel permeation column to remove unreacted polypeptide and residual reagents.

[0058] In some embodiments, CDAP can be made up at 100 mg/ml in acetonitrile and stored at -20°C for up to 1 month. CDAP can be slowly pipetted into a vortexed solution of polysaccharide in water (rapid addition of the organic co-solvent precipitates the polysaccharide) and 20 s later a volume of aqueous 0.2M triethylamine (TEA) equal to volume of CDAP used can be added. At 2.5 min, a large molar excess of a 0.5 M hexanediamine solution in either 0.1 M sodium borate at pH 9.3 or 0.75 M HEPES at pH 7.5, can be added. The reaction mixture can be allowed to stand overnight at 4°C, can be desalted on either a P-6 DG or a P-6 cartridge, can be equilibrated with saline and then can be further dialyzed into saline. The extent of derivatization with hexanediamine can be determined using a TNBS assay for primary amines; absorbance can be measured at 366nm, using an extinction coefficient of 11000 M^{-1}. Polysaccharide concentrations can be determined as described by Monsigny et al., using the corresponding polysaccharide as the standard.

[0059] In some embodiments, polysaccharide can be solubilized in saline at 10 mg/ml. At t = 0, CDAP (100 mg/ml in acetonitrile) can be added (1 mg CDAP/ mg polysaccharide) while vortexing. At 30 s, 0.2 M TEA can be added (10 µl/mg polysaccharide) to raise the pH. At 2.5 min the pH can be readjusted to the coupling pH and the amine or hydrazide reagent or the polypeptide can be added, in the appropriate buffer. After an overnight reaction, the solution can be dialyzed and/or desalted on a P6DG column. Polysaccharide polypeptide conjugates can be fractionated on an S400HR column, which can be equilibrated with either saline or PBS. HPLC can be performed on HPLC size exclusion columns using a Waters 626 pump, monitored at 280 nm and with a refractive index detector. Samples can be filtered before chromatography using a Millex Ultrafree MC device.

[0060] In some embodiments, for low pH coupling, amines on polypeptide can be functionalized with hydrazides using the two-step method as follows. Five mg of polypeptide at 12.5 mg/ml in 0.19 M HEPES, 2.5 mM EDTA, pH 7.3 can be iodoacetylated by the addition of a 15-fold molar excess of SIA over moles of polypeptide. After 2 h reaction in the dark, the solution can be desalted on a 1 x 15 cm P6DG column, can be equilibrated with 2 mM MES, 0.2 M NaCl, pH 5.9 and the void volume fractions, which can be concentrated with a Centricon 50 device. DTPH (30 µl) at 0.1 M in water, can be reduced to 3-mercapto-propionyl hydrazide by addition of 25 µl 0.1 M TCEP in water, and can then be added to the

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iodoacetylated polypeptide with a pH that can be adjusted to about 5. After a 2 h reaction, reagent can be removed by desalting again and concentrating with a Centricon 50 device to 10 mg/ml. TNBS can be used to determined number of hydrazide per moles of polypeptide. To couple the hydrazide-derivatized polypeptide, 3 mg of polysaccharide at 10 mg/ml in saline can be activated by the addition of 15 µl CDAP at 100 mg/ml in acetonitrile, followed 30 s later by 30 µl of 0.2 M TEA. At 2.5 min, the pH can be reduced to 5.5 by the addition of 100 µl 1 M MES, pH 5.5 and the activated polysaccharide can be added to 3.8 mg of the polypeptide-hydrazide. After an overnight reaction at 4°C, the reaction can be quenched by the addition of 50 µl 1 M ethanolamine. The solution can be applied to an S400HR column, which can be equilibrated with PBS, and the void volume fractions can be pooled. Sterile filtration can be done by passage through a Millex GB 0.2 µm filter.

[0061] In some embodiments, CDAP can be used to conjugate a polysaccharide and CRM_{197}. The polysaccharide (10 mg) can be dissolved in 1.0 mL of 0.1M sodium borate buffer pH 9.0 and can be activated by addition of 50 µl of a solution of CDAP dissolved in acetone:water (6:4). Immediately, the pH can be adjusted and maintained at 9.0 by continuous dropwise addition of a 0.1 M NaOH solution. The mixture can be stirred for 5 min and then a volume of 1.4 mL of 0.05 M HCl can be added, stirred for a while and then left to rest during 1 ½ min more. The mixture can be rapidly passed through a PD-10 column equilibrated in 0.1 M sodium bicarbonate buffer pH 8.3. An aliquot of this can be used to determine cyanate ester formation and the rest of the preparation can be immediately used for coupling purposes. Polypeptide (15 mg) can be dissolved in 1.0 mL of 0.1 M $NaHCO_3$ buffer pH 8.3 and added to the activated polysaccharide solution. The mixture can be gently stirred during 8 h at room temperature. A volume of 1.0 mL 0.1 M Tris-HCl buffer pH 8.6 can be added to block any remaining activation sites on the unreacted polysaccharide and incubated during 1 h more. The final solution can be lyophilized and stored until used for the purification procedure. The progress of the conjugation can be analyzed by high-performance size exclusion chromatography (HPSEC) using a Zorbax GF-250 column and monitored at 280 nm and 210 nm. For example, aliquots of the reaction mixture can be taken at 10 min and at every hour up to 4 h. The column can be equilibrated in 0.025 M phosphate buffer pH 7.0 supplemented with 0.13 M NaCl and be calibrated with the following proteins: thyroglobulin(669 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), BSA (67 kDa), ovalbumin (43 kDa), and ribonuclease (13.7 kDa). The void volume can be determined with Blue dextran. Samples (10 µl aliquots) can be injected into the column and a flow rate of 0.5 mL/min can be used. Prior and after the analysis the column can be washed with the equilibration buffer.

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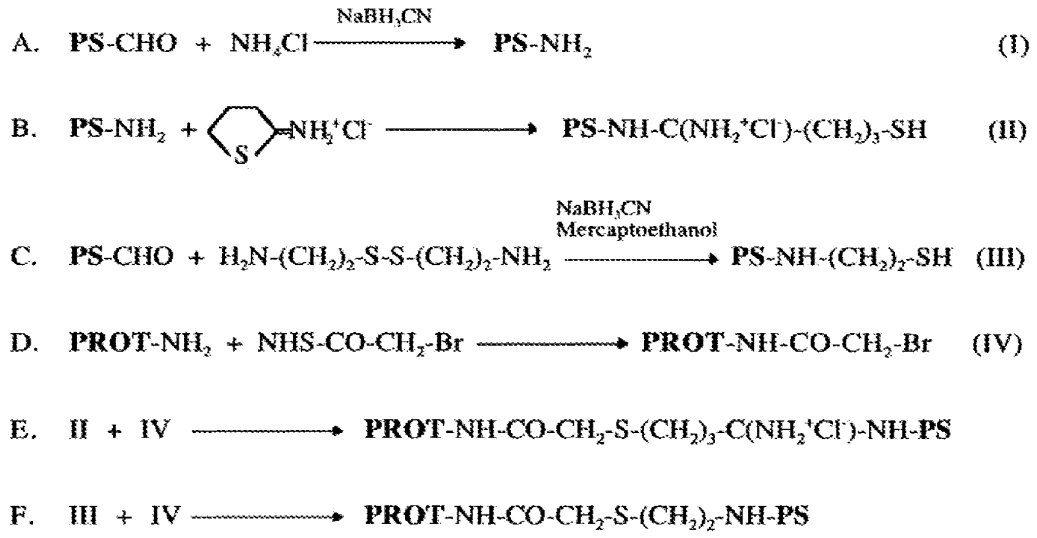
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[0062] In some embodiments, the polysaccharide can be activated via CDAP chemistry in a CDAP solution. The polysaccharide can be mixed with CDAP solution, which can then be incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} can be added to the activated polysaccharides. The reaction can then be quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture can be filtered, diafiltered, and concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates can then be purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates can be pooled, concentrated, and then filtered through a 0.2 μm filter. The product can be stored at 2-8°C.

[0063] As another example, the capsular polysaccharide or immunogenic fragment thereof can activated by periodate oxidation. Sodium periodate oxidizes diols (two adjacent carbons with hydroxyl groups) can be converted into aldehydes (C=O) and in the process breaks C-C bonds. Thus, depending upon the structure, periodate activation can fragment a capsular polysaccharide or immunogenic fragment thereof and open the ring structures of sugars. When the diol is within a ring, the ring sugar can be opened possibly altering the polysaccharide conformation. When the diol is in a glycerol or ribitol side chain, the side chain can disappear. The reactive aldehyde groups can then condense with free amino groups on the polypeptide in the presence of sodium cyanoborohydride to form a stable secondary amine. Condensation of the aldehyde with the epsilon amino group on lysine can be a slow process and can take a few days. The remaining aldehyde groups can then be quenched using sodium borohydride, which can convert them back to hydroxyls.

[0064] Another example of coupling a polypeptide to a polysaccharide is based on thioether bonding of polysaccharides activated with 2-iminothiolane (2-IT), or alternatively, with cystamine, to bromoacetylated polypeptides such as tetanus toxoid.

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Schematic I

[0065] The above Schematic I represents end-point coupling of polysaccharides (PS) to proteins (PROT) through thioether bond. (A) Amination of polysaccharide through reducing terminal using ammonium chloride in the presence of cyanoborohydride ion. (B) Thiolation of aminated polysaccharide (I) with 2-iminothiolane (2-IT). (C) Alternative thiolation of polysaccharide through reductive amination with cystamine with concomitant cleavage of disulfide bond. (D) Bromoacetylation of amino groups of protein using N-hydroxysuccimidyl bromoacetate. (E) Coupling of polysaccharide derivatized with 2-IT (II) to bromoacetylated protein (IV). (F) Alternative coupling of polysaccharide derivatized with cystamine (III) to bromocacetylated protein (IV).

[0066] As another example, the immunogenic saccharide-polypeptide conjugate can be produced using bacteria, such as *Escherichia coli* (*E. coli*). The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially embedded in the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide chemically cross-linked to the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially chemically cross-linked to the polypeptide. A capsular polysaccharide or fragment of the capsular polysaccharide of a serotype from *S. pneumoniae* can be conjugated to a polypeptide by chemical or biological synthesis. One saccharide can be conjugated to one polypeptide. Two or more saccharides can be conjugated to one polypeptide. The saccharides can be the same serotype. The saccharides can be different serotypes. One capsular polysaccharide or immunogenic fragment thereof can be conjugated to one polypeptide. Two or more capsular polysaccharides or fragments thereof can be conjugated to one polypeptide. The capsular

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polysaccharides or fragments thereof can be the same serotype. The capsular polysaccharides or fragments thereof can be different serotypes.

[0067] After conjugation, the polysaccharide polypeptide conjugate can be purified. For example, different chromatographic strategies can be used to purify a polysaccharide-polypeptide conjugate: SEC (using a column packed with Sepharose CL-6B), a combination of ion-exchange chromatography (DEAE-Sepharose) can be followed by HPSEC (Sepharose CL-6B and hydrophobic interaction chromatography (HIC) (column packed with Sephadex LH-20). For HPSEC purification on a Sepharose CL-6B column, an amount of 4.0 mg of the lyophilized mixture containing the polysaccharide polypeptide conjugate, free polysaccharide and free polypeptide, can be dissolved in 0.1 M formic acid buffer (ammonium salt) pH 6.0 and can be applied to the column (60 cm x 4.0 cm) equilibrated with the same buffer, at a flow of 0.5 mL/min. Isocratic elution can be performed with the buffer, and the process can be continuously monitored using a differential refractometric detector. Fractions can be collected, can be lyophilized, and can be used for further analysis. For the second strategy, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 500 μL of 0.1 M phosphate-buffered saline (PBS) pH 7.0 and can be applied to the DEAE-Sepharose column, previously equilibrated in the same buffer. The pass-through of the column can be collected and after washing with the buffer, elution can be performed using a gradient (0.25-1.0 M) of NaCl. The presence of the polysaccharide can be analyzed in each fraction by the Phenol sulfuric technique. The eluted fractions can be pooled, can be lyophilized, can be resuspended in 0.1 M ammonium formate buffer pH 6.0, and can be applied to a Sepharose CL-6B column. Fractions of 5 mL can be collected, can be dialyzed, can be lyophilized, and can be analyzed for protein and carbohydrate contents. In a third chromatographic approach, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 1.0 mL of distilled water and applied to a column packed with Sephadex LH-20 previously equilibrated with distilled water, at a flow rate of 0.5 mL/min. Elution can be performed isocratically with distilled water, and the process can be monitored as described above. Fractions can be collected, can be lyophilized, and can be used for further analysis.

[0068] Furthermore, 'click' chemistry can be used to conjugate a saccharide with a polypeptide as disclosed in US Patent No. 8,753,645 and in Hu et al., Towards the next generation of biomedicines by site selective conjugation, *Chem. Soc. Rev.,* 2016, 45, 1691. 'Click' chemistry can be used to conjugate modified CRM_{197} or CRM_{197} with non-natural amino acids to polysaccharides.

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Pharmaceutical compositions

[0069] The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition. The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition in unit dose form. The saccharide-polypeptide conjugates described herein can be in the form of a vaccine. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can be in unit dose form and can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate wherein the capsular polysaccharide or immunogenic fragment thereof of the saccharide-polypeptide conjugate can be one serotype. In another embodiment, a pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the capsular polysaccharides or immunogenic fragments thereof can be from different serotypes, creating a mixture of different immunogenic saccharide-polypeptide conjugates. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the polypeptide is the same polypeptide in all the immunogenic saccharide-polypeptide conjugates. In other embodiments, a pharmaceutical composition can comprise immunogenic saccharide-polypeptide conjugates wherein the polypeptide(s) in the saccharide-polypeptide conjugates are a mixture of different polypeptide(s).

[0070] For example, a pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide,

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fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least eight immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31,34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes

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selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, , 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide

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conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide

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conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 34F, 31, 33B,34, 35F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 20B, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, 38 or

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Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at

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least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes

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comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at

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least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates

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comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising

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serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20

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(comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F,

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24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. The immunogenic saccharide-polypeptide conjugate(s) can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include excipients that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include adjuvants that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The pharmaceutical composition can be administered to one of a plurality of humans, which elicits at least one of the following side effects in less than about 5%, 10%, 20% or 30% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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[0071] In other embodiments, a pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide is from *N. meningitidis*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *S. enterica*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *H. influenzae*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *Neisseria meningitidis*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *S. enterica*. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *H. influenzae*.

[0072] The polypeptide of any of the plurality of saccharide-polypeptide conjugates can be the same polypeptide. For example, the polypeptide of any of the plurality of saccharide-polypeptide conjugates can be CRM_{197}. The plurality of saccharide-polypeptide conjugates can comprise a mixture of different polypeptides. For example, the plurality of saccharide-polypeptide conjugates can comprise a mixture of CRM_{197}, tetanus toxoid, a diphtheria toxoid, or any fragment thereof.

[0073] Any pharmaceutical composition described herein can be in unit dose form. Additionally, the pharmaceutical composition can further comprise an adjuvant. In some embodiments, the adjuvant can be an aluminum-cation comprises adjuvant. In other embodiments, the adjuvant can be aluminum phosphate, aluminum sulfate, or aluminum hydroxide. More examples of suitable adjuvants for use in the pharmaceutical composition include: aluminum-based salts and variants thereof; emulsions (either water-in-oil or oil-in-water) and variants thereof, *e.g.*, Freund's Incomplete Adjuvant, MF59® (Glaxosmithkline plc); PRR ligands and variants thereof, *e.g.*, pathogen associated molecular patterns (PAMPs); TLR3 and RLR ligands or variants thereof, *e.g.*, Synthetic analogs of dsRNA, poly(I:C), etc.; TLR4 ligands or variants thereof, *e.g.*, bacterial flagellin, Glucopyranosyl Lipid Adjuvant (GLA), monophosphoryl lipid A, etc.; TLR5 ligands or variants thereof, *e.g.*, imiquimod, gardiquimod, R848, etc.; TLR9 ligands or variants thereof, *e.g.*, Oxydeoxynucleotides containing CpG motifs (CpG ODNs such as ODN1826 and ODN2006); NOD2 ligands or variants thereof, *e.g.*, fragments of bacterial cell walls (such as muramyl dipeptide [MDP]); saponins including synthetic derivatives or variants thereof, e.g., QS21, TQL1055, etc.; or any combination of above. An adjuvant can be 3M-052.

[0074] A pharmaceutical composition can further comprise an excipient. An excipient can be Acacia, Acesulfame Potassium, Acetic Acid, Glacial Acetone, Acetyltributyl Citrate,

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Acetyltriethyl Citrate, Agar, Albumin, Alcohol, Alginic Acid, Aliphatic Polyesters, Alitame, Almond Oil, Alpha Tocopherol, Aluminum Hydroxide Adjuvant, Aluminum Oxide, Aluminum Phosphate Adjuvant, Aluminum Stearate, Ammonia Solution, Ammonium Alginate, Ascorbic Acid, Ascorbyl Palmitate, Aspartame, Attapulgite, Bentonite, Benzalkonium Chloride, Benzethonium Chloride, Benzoic Acid, Benzyl Alcohol, Benzyl Benzoate, Boric Acid, Bronopol, Butylated Hydroxyanisole, Butylated Hydroxytoluene, Butylparaben, Calcium Alginate, Calcium Carbonate, Dibasic Anhydrous Calcium Phosphate, Dibasic Dihydrate Calcium Phosphate, Tribasic Calcium Phosphate, Calcium Stearate, Calcium Sulfate, Canola Oil, Carbomer, Carbon Dioxide, Carboxymethylcellulose Calcium, Carboxymethylcellulose Sodium, Carrageenan, Castor Oil, Hydrogenated Castor Oil, Microcrystalline Cellulose, Powdered Cellulose, Silicified Microcrystalline Cellulose, Cellulose Acetate, Cellulose Acetate Phthalate, Ceratonia, Cetostearyl Alcohol, Cetrimide, Cetyl Alcohol, Cetylpyridinium Chloride, Chitosan, Chlorhexidine, Chlorobutanol, Chlorocresol, Chlorodifluoroethane (HCFC), Chlorofluorocarbons (CFC), Chloroxylenol, Cholesterol, Citric Acid Monohydrate, Colloidal Silicon Dioxide, Coloring Agents, Copovidone, Corn Oil, Cottonseed Oil, Cresol, Croscarmellose Sodium, Crospovidone, Cyclodextrins, Cyclomethicone, Denatonium Benzoate, Dextrates, Dextrin, Dextrose, Dibutyl Phthalate, Dibutyl Sebacate, Diethanolamine, Diethyl Phthalate, Difluoroethane (HFC), Dimethicone, Dimethyl Ether, Dimethyl Phthalate, Dimethyl Sulfoxide, Dimethylacetamide, Disodium Edetate, Docusate Sodium, Edetic Acid, Erythorbic Acid, Erythritol, Ethyl Acetate, Ethyl Lactate, Ethyl Maltol, Ethyl Oleate, Ethyl Vanillin, Ethylcellulose, Ethylene Glycol Palmitostearate, Ethylene Vinyl Acetate, Ethylparaben, Fructose, Fumaric Acid, Gelatin, Glucose, Liquid, Glycerin, Glyceryl Behenate, Glyceryl Monooleate, Glyceryl Monostearate, Glyceryl Palmitostearate, Glycofurol, Guar Gum, Hectorite, Heptafluoropropane (HFC), Hexetidine, Hydrocarbons (HC), Hydrochloric Acid, Hydroxyethyl Cellulose, Hydroxyethylmethyl Cellulose, Hydroxypropyl Cellulose, Low-substituted Hydroxypropyl Cellulose, Hydroxypropyl Starch, Hypromellose, Hypromellose Acetate Succinate, Hypromellose Phthalate, Imidurea, Inulin, Iron Oxides, Isomalt, Isopropyl Alcohol, Isopropyl Myristate, Isopropyl P,almitate, Kaolin, Lactic Acid, Lactitol, Anhydrous Lactose, Monohydrate Lactose, Spray-Dried Lactose, Lanolin, Lanolin Alcohols, Hydrous Lanolin, Lauric Acid, Lecithin, Leucine, Linoleic Acid, Macrogol, Hydroxystearate Magnesium Aluminum Silicate, Magnesium Carbonate, Magnesium Oxide, Magnesium Silicate, Magnesium Stearate, Magnesium Trisilicate, Malic Acid, Maltitol, Maltitol Solution, Maltodextrin, Maltol, Maltose, Mannitol, Medium-chain Triglycerides, Meglumine, Menthol,

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Methylcellulose, Methylparaben, Mineral Oil, Light Mineral Oil, Mineral Oil and Lanolin Alcohols, Monoethanolamine, Monosodium Glutamate, Monothioglycerol, Myristic Acid, Neohesperidin Dihydrochalcone, Nitrogen, Nitrous Oxide, Octyldodecanol, Oleic Acid, Oleyl Alcohol, Olive Oil, Palmitic Acid, Paraffin, Peanut Oil, Pectin, Petrolatum, Petrolatum and Lanolin Alcohols, Phenol, Phenoxyethanol, Phenylethyl Alcohol, Phenylmercuric Acetate, Phenylmercuric Borate, Phenylmercuric Nitrate, Phosphoric Acid, Polacrilin Potassium, Poloxamer, Polycarbophil, Polydextrose, Polyethylene Glycol, Polyethylene Oxide, Polymethacrylates, Poly(methyl vinyl ether/maleic anhydride), Polyoxyethylene Alkyl Ethers, Polyoxyethylene Castor Oil Derivatives, Polyoxyethylene Sorbitan Fatty Acid Esters, Polyoxyethylene Stearates, Polyvinyl Acetate Phthalate, Polyvinyl Alcohol, Potassium Alginate, Potassium Benzoate, Potassium Bicarbonate, Potassium Chloride, Potassium Citrate, Potassium Hydroxide, Potassium Metabisulfite, Potassium Sorbate, Povidone, Propionic Acid, Propyl Gallate, Propylene Carbonate, Propylene Glycol, Propylene Glycol Alginate, Propylparaben, 2-Pyrrolidone, Raffinose, Saccharin, Saccharin Sodium, Saponite, Sesame Oil, Shellac, Simethicone, Sodium Acetate, Sodium Alginate, Sodium Ascorbate, Sodium Benzoate, Sodium Bicarbonate, Sodium Borate, Sodium Chloride, Sodium Citrate Dihydrate, Sodium Cyclamate, Sodium Hyaluronate, Sodium Hydroxide, Sodium Lactate, Sodium Lauryl Sulfate, Sodium Metabisulfite, Dibasic Sodium Phosphate, Monobasic Sodium Phosphate, Sodium Propionate, Sodium Starch Glycolate, Sodium Stearyl Fumarate, Sodium Sulfite, Sorbic Acid, Sorbitan Esters (Sorbitan Fatty Acid Esters), Sorbitol, Soybean Oil, Starch, Pregelatinized Starch, Sterilizable Maize Starch, Stearic Acid, Stearyl Alcohol, Sucralose, Sucrose, Compressible Sugar, Confectioner's Sugar, Sugar Spheres, Sulfobutylether b-Cyclodextrin, Sulfuric Acid, Sunflower Oil, Hard Fat Suppository Bases, Talc, Tartaric Acid, Tetrafluoroethane (HFC), Thaumatin, Thimerosal, Thymol, Titanium Dioxide, Tragacanth, Trehalose, Triacetin, Tributyl Citrate, Triethanolamine, Triethyl Citrate, Vanillin, Hydrogenated Vegetable Oil, Water, Anionic Emulsifying Wax, Carnauba Wax, Cetyl Esters Wax, Microcrystalline Wax, Nonionic Emulsifying Wax, White Wax, Yellow Wax, Xanthan Gum, Xylitol, Zein, Zinc Acetate, or Zinc Stearate.

[0075] A pharmaceutical composition can further comprise a surfactant or emulsifier, or a combination thereof. A surfactant can be polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. In some embodiments, polysorbate can be polysorbate 80. In some embodiments, polymer glycol can be polyethylene glycol. A surfactant can be TWEEN. A pharmaceutical composition can comprise a specific polyethylene glycol based upon its molecular weight. A pharmaceutical composition can comprise a preservative. A

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pharmaceutical composition can comprise a salt. The salt is an inorganic salt. A pharmaceutical composition can further comprise an anti-fungal compound or a salt thereof. An antifungal compound can be thimerosal, phenol, benzethonium chloride, or 2-phenoxyethanol. A pharmaceutical composition can further comprise a chelating agent. A chelating agent can be ethylenediaminetetraacetic acid (EDTA). A pharmaceutical composition can further comprise a buffering agent. The buffering agent is succinate buffer agent, tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, phosphate buffering agent, sodium phosphate buffering agent, or a potassium phosphate buffering agent. A pharmaceutical composition can further comprise sterile water. When the pharmaceutical composition can be administered intramuscularly to a human, the pharmaceutical composition can elicit an immune response that lasts from about 1 week to about 30 years. A pharmaceutical composition retains at least about 60% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 70% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 90% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0076] The polysaccharide-polypeptide conjugates as described herein can be adjuvanted. Suitable adjuvants can include an aluminum hydroxide gel (alum) or aluminum phosphate, but can also be a salt of calcium, magnesium, iron or zinc, or can be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized polysaccharides, or polyphophazenes. An adjuvant can be 3M-052. When adjuvanted with aluminum salts, the ratio of aluminum salt to polysaccharide can be less than 10:1 (w/w), less than 8:1(w/w), or more than 2:1(w/w) in a vaccine dose or formulation.

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[0077] The amount of polypeptide in a vaccine dose can be selected as an amount which can induces an immunoprotective response without significant adverse effects. A dose or formulation can comprise 0.1-100 μg of polysaccharide and/or for a polysaccharide polypeptide conjugate 0.1-50 μg of polysaccharide. A polysaccharide-polypeptide conjugate can comprise 1-10 μg of polysaccharide, 1-5 μg of polysaccharide, or 2-5 μg of polysaccharide. The content of polypeptide in the vaccine can be in the range of 1-100 μg of polypeptide, 5-50 μg of polypeptide, or 5-25 μg of polypeptide.

[0078] The vaccine dosage can be in the range of 1-5 μg of polysaccharide per polysaccharide polypeptide conjugate delivered intramuscularly as a single 0.5 mL injection. The vaccine dosage can comprise aluminum phosphate (1 mg/mL) and 0.01% polysorbate 80.

Methods of Use

[0079] The saccharide-polypeptide conjugates as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be used in a vaccine. The vaccine can be administered to a subject. The vaccine can be used as a prophylatic for disease caused by *S. pneumoniae*, such as meningitis, pneumonia, and severe invasive diseases associated with pneumococcal infection. The subject can be a human. The subject or human can be a subject or human in need thereof.

[0080] The saccharide-polypeptide conjugate can be administered by intramuscular injection. The saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The saccharide-polypeptide conjugate can be administered via a suppository. The saccharide-polypeptide conjugate can be administered intranasally. The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the saccharide-polypeptide conjugate.

[0081] The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered by intramuscular injection. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary,

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respiratory or genitourinary tracts. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered intranasally. The pharmaceutical composition can be administered intramuscularly and can elicit an immune response that lasts from 1 week to 30 years. The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the pharmaceutical composition comprising the saccharide-polypeptide conjugate.

[0082] For example, a pharmaceutical composition administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, or of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least

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fourteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eighteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). For example, a pharmaceutical composition administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

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23, or of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fourteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eighteen serotypes selected from

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the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising 20A and 20B).A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition administered to a subject can

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comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F,

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34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F,

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34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B) or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B).A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise

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administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition administered to a subject can comprise can comprise a

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plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.

[0083] Each dosage can be formulated in 0.5 mL. The formulation can comprise normal saline. The formulation can have a pH of 6.5. The formulation can be a pH from 6.3 to 6.7 or from 6.2 to 6.8. The formulation can have a pH of 5.8. The formulation can be a pH from 5.5 to 6.1 or from 5.6 to 6.0. The formulation can comprise 0.001% to 0.01% polysorbate 80.

[0084] Two different pharmaceutical compositions can be administered to a subject. The first pharmaceutical composition can be administered to the subject at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years before or at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years after an administration to the subject a second pharmaceutical composition. The first pharmaceutical composition can be administered to a subject who is at least 4 weeks old, 6 weeks old, 12 weeks old, 24 weeks old, 6 months old, 8 months old, 10 months old, 12 months old, 14 months old, 15 months old, 16 months old, 17 months old, 18 months old, 19 months old, 20 months old, 21 months old, 22 months old, 23 months old, 2 years old or older. The first pharmaceutical composition can be administered to a subject with an age from 4 weeks to 2 years old. The first pharmaceutical composition can be administered to a subject with an age from 18 years old to 100 years old. The first pharmaceutical composition can be administered to a subject with an age of 100 years old or older. In some embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide

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conjugate comprising a polypeptide that has at less than 70% length homology, 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0085] In some embodiments, the first pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). In some embodiments, the first pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); or a combination thereof. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B). The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F,34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B).The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A,

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11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the

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serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. In other embodiments, the first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. When administering any of the first pharmaceutical compositions described above, the first pharmaceutical composition can be distinct from the second pharmaceutical composition administered to a subject.

[0086] In some embodiments, the second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and

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Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates

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comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B,

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33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct.

[0087] In other embodiments, the first pharmaceutical composition and the second pharmaceutical composition can be administered at least about 4 weeks apart, at least 6 about weeks apart, at least 2 about months apart, at a least about 3 months apart, at least about 4 months apart, at least about 5 months apart, at least 6 months apart, at least about 7 months apart, at least about 8 months apart, at least about 9 months apart, at least about 10 months apart, at least 11 about months apart, at least about 1 year apart, at least about 1.5 years apart, at least about 2 years apart, at least about 5 years apart, at least about 10 years apart, at least about 15 year, at least about 20 years apart, or at least about 30 years apart.

[0088] In some embodiments, a serotype of the first pharmaceutical composition can be from a different bacteria than the serotype of the second pharmaceutical composition. For example, the serotype of the first composition can be from *Streptococcus pneumoniae*, and the serotype of the second composition can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. The serotype of the first pharmaceutical composition can be from

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Neisseria meningitidis, *Salmonella enterica*, or *Haemophilus influenzae*, and the serotype of the second pharmaceutical composition can be from *Streptococcus pneumoniae*.

[0089] In some embodiments, a serotype of the first pharmaceutical composition can be from the same bacteria as a serotype for the second pharmaceutical composition. For example, the first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F 38 and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and

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Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*,

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and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F,

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35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

[0090] Different serotypes of *S. pneumoniae* can be more pervasive or endemic in different geographical areas. For example, a certain group of serotypes can commonly cause pneumococcal disease in the United States and Canada. This group of serotypes can be different for Europe, the Middle East, North Africa, Japan and Korea. For example, administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Europe, the Middle East, and/or North Africa. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 15C, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Japan and/or Korea. Additionally, invasive clinical disease can be caused by serotype 20B (as opposed to serotype 20A). The subject can be a human who resides in the specific geographical location as indicated above, or can be a human who is visiting the specific geographical location as indicated above.

[0091] A method of administration can comprise administering a first saccharide-polypeptide conjugate composition to a subject; administering a second saccharide-polypeptide conjugate composition to the subject at least four weeks after administering the first saccharide-

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polypeptide conjugate composition; wherein the first saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a second polypeptide of the second saccharide-polypeptide conjugate composition; and wherein the first saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. In some embodiments, the first serotype is from the same bacteria as the second serotype. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition comprises a saccharide-polypeptide conjugate comprising a polypeptide that has at less than about 70% length homology, about 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0092] In other embodiments, the conducting of any of the methods described herein may not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

Saccharide-polypeptide Conjugate Kits

[0093] The saccharide-polypeptide conjugates and pharmaceutical compositions described herein can be contained in a kit. The kit can further comprise instructions for use. The saccharide-polypeptide conjugates and/or pharmaceutical compositions can be stored in a container. The container can be a syringe. The container can be a vial. The vial can be a 2 mL borosilicate glass vial. The vial can be 3 mL Vials USP Type I glass, non-siliconized. The top can be an aluminum crimp top. The container can be a vial with a rubber septum or a septum made of flexible, reclose-able material. The container can be a vial with a stopper. The stopper can be a non-siliconized stopper. The vial can have a fill volume of 0.6 mL. The extractable volume can be 0.5 mL.

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EXAMPLES

[0094] The following examples are included to further describe some aspects of the present disclosure, and should not be used to limit the scope of the embodiments described herein.

EXAMPLE 1

Method of Establishing a Cell Bank

[0095] This example shows the establishment of a cell bank for a *S. pneumoniae* strain for use in producing polysaccharides for a polysaccharide-CRM_{197} conjugate vaccine. Any strain that produces the polysaccharides of **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are established as a cell bank by this method. A *S. pneumoniae* strain is provided by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG). The cell bank process is depicted in **FIGURE 2**. A vial of a strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol and then is dispensed into cryogenic vials. The cell bank is contained in the cryogenic vials, which are stored at -70°C until use.

[0096] *S. pneumoniae* strains that produce serotype 6C polysaccharide was acquired by the University of Alabama, Birmingham (UAB) for establishing a 6C cell bank. The cell bank process is depicted in **FIGURE 2**. For 6C polysaccharide serotype, a vial of the strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol and then is dispensed into cryogenic vials. The cell bank for each strain are contained in the cryogenic vials, which are stored at -70°C until use.

[0097] *S. pneumoniae* strains that produce serotype 8 polysaccharide, serotype 10A polysaccharide, serotype 11A polysaccharide, serotype 12F polysaccharide, serotype 22F

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polysaccharide, or serotype 33F polysaccharide were acquired by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG) for establishing a cell bank. The cell bank process is depicted in **FIGURE 2**. For each polysaccharide serotype, a vial of the strain of *S. pneumoniae* was seeded and then was grown overnight on an agar plate. A single colony was picked from this plate and streaked onto a new agar plate for overnight growth. This process was repeated three times to purify the strain before a flask was seeded and was incubated. After allowing the seeded flask sufficient time for growth, the flask contents were harvested by centrifugation. The centrifugation product was resuspended in a medium of about 20% glycerol and then was dispensed into cryogenic vials. The cell bank for each strain were contained in the cryogenic vials, which were stored at -70°C until use.

EXAMPLE 2

Method of Isolating and Purifying Polysaccharides

[0098] This examples shows how polysaccharides are isolated and purified from cell banks for use in a polysaccharide-CRM_{197} conjugate vaccine. Any of the polysaccharides of **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are isolated and purified by this method. This process for the isolation and purification of polysaccharides is shown in **FIGURE 4**. A vial of a cell bank produced as described in **EXAMPLE 1** is used to inoculate a flask containing an appropriate culture medium and incubated overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 μm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

[0099] A cell bank of an *S. pneumoniae* strain that produces serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F was used to inoculate a flask containing the appropriate culture medium and incubated overnight. This culture was used to seed a new culture for fermentation in a fermenter. The culture was allowed to grow under

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the appropriate conditions for the specific strain. After fermentation, the cells were inactivated and then were treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris was removed by centrifugation, which was followed by a depth filter step through a 0.45 μm filter and the cell free broth was collected. The product was then purified and the polysaccharide size was determined. Quality control tests were performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

EXAMPLE 3

Method of CRM_{197} production

[0100] This example shows the production of CRM_{197} (SEQ ID NO:1) for use in saccharide polypeptide vaccines, which is depicted in **FIGURE 3.** A vial from a working cell bank of CRM_{197} was seeded into a flask containing the appropriate culture medium and incubated overnight. The fermentation culture was centrifuged and the contents were harvested, were clarified, and then were filtered through a 0.2 μm filter. The filtrate was concentrated about ten-fold, and then was diafiltered to exchange the buffer and remove contaminants. The protein was further purified by microfiltration using an appropriate pore size membrane. Then the filtrate was further purified by anion exchange chromatography, followed by ammonium sulphate precipitation, and filtration (0.45 μm). A final purification using hydrophobic chromatography was conducted, and the highly purified CRM_{197} protein was concentrated and diafiltered, and then filtered through a 0.2 μm filter. The product was stored at -70°C until use in conjugation with a polysaccharide, such as any polysaccharide listed in **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)). Quality control tests were performed on the product to assess concentration (BCA for Lowry NLT 15 mg/mL), purity (SDS-PAGE gel with a target of ≥ 95% pure), confirming CRM_{197} identification (using a monoclonal antibody), assessing size (SDS-Page with a target of ~58 kDa), assessing endotoxin level (LAL kit with target of <1 IU/μg of protein), assessing lack of toxicity (Vero Cytotoxicity or DT enzyme test; non-lethal in Guinea Pigs), assessing pH (meter, target of 6.5 ± 0.2), and assessing appearance (visually, target for clear liquid).

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EXAMPLE 4

Method of conjugation

[0101] This example shows the conjugation of a capsular polysaccharide to a polypeptide via CDAP conjugation chemistry. The polysaccharide are isolated as described in **EXAMPLE 2** and are thawed. The polypeptide is chosen from any of the following: diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof. The polysaccharide is activated via CDAP chemistry in a CDAP solution. The polypeptide is added to the activated polysaccharides, and then the conjugation reaction is quenched. The reaction mixture is filtered, is diafiltrated, and is concentrated. The polysaccharide polypeptide conjugates are then separated using gel filtration chromatography. These conjugates are then concentrated for the final polysaccharide polypeptide conjugate.

EXAMPLE 5

Method of Polysaccharide-CRM_{197} Conjugation

[0102] This example shows the conjugation of a capsular polysaccharide to CRM_{197} via CDAP conjugation chemistry. The isolated polysaccharide is any polysaccharide as described in **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)), or in **EXAMPLE 2**, and CRM_{197} is as described in **EXAMPLE 3**. The conjugation process is depicted in **FIGURE 5**. The polysaccharide and CRM_{197} are thawed. The polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} is added to the activated polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the

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polysaccharide-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The product is stored at 2-8°C.

[0103] The isolated polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F as described in **TABLE 1** and **EXAMPLE 2**, and CRM_{197} as described in **EXAMPLE 3** were thawed. The polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F were mixed with CDAP solution, which was then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} was added to the activated polysaccharides. The reaction was then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture was filtered, was diafiltered, and was concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates were then purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates were pooled, were concentrated, and then were filtered through a 0.2 μm filter. The product is stored at 2-8°C.

EXAMPLE 6

Combinations of Polysaccharide-CRM_{197} Conjugates in a Vaccine Composition

[0104] This example shows combinations of polysaccharides that are used in a polysaccharide-CRM_{197} conjugate vaccine. Each polysaccharide serotype is individually conjugated to a CRM_{197} to produce the polysaccharide CRM197 conjugate. The polysaccharide-CRM_{197} conjugates for each serotype are combined into vaccine composition. See the below **TABLE 2** for exemplary polysaccharide-CRM_{197} conjugate serotype combinations.

TABLE 2. Combinations of Polysaccharides in Polysaccharide-CRM_{197} Conjugate Vaccines

List of Polysaccharide Serotype Combinations
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33B, and 33F
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B

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List of Polysaccharide Serotype Combinations
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33B, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33B, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 23A, 23B, 31, 33A, 34, 35F, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 31, 33F, 33A, 34, 35B, 35F, and 38
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 20B, and 23F
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 20B,22F, 23A, 23B, 33B, and 33F
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B

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List of Polysaccharide Serotype Combinations
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 20B, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 20B, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33B, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 20B, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33B, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 31, 33A, 34, 35F, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 20B,

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List of Polysaccharide Serotype Combinations
22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 33A, 34, 35B, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B
6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B

EXAMPLE 7

Formulation of Polysaccharide-CRM_{197} Conjugate Vaccine

[0105] This example shows a formulation for a polysaccharide-CRM_{197} conjugate vaccine. Any combination of polysaccharide-CRM_{197} conjugates from **EXAMPLE 5** or the combinations listed in **EXAMPLE 6** are used in the vaccine composition. In addition to the polysaccharide-CRM_{197} conjugates, the vaccine is composed of normal saline and 0.01% Polysorbate 80, with a pH of 6.5 ± 0.2. This formulation of the polysaccharide CRM_{197} vaccine composition is stored in 2 mL glass vials with an aluminum crimp top and stopper at 2-8°C. The vial is filled to a volume of 0.60 mL to allow for an extractable volume of 0.50 mL.

EXAMPLE 8

Process for Formulating a Polysaccharide-CRM_{197} Conjugate Vaccine

[0106] This example shows the formulating process for a polysaccharide CRM_{197} conjugate vaccine. The polysaccharide CRM_{197} conjugates are prepared as described in the above Examples. The formulation process is depicted in **FIGURE 6**. 0.9% w/v sterile saline is added to a formulation container. Each type of conjugate is separately sterilized and the appropriate volume of each monovalent bulk polysaccharide-CRM_{197} conjugate is added

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(volume is based on the polysaccharide concentration) to the formulation container one at a time. The pH is adjusted to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH. Optionally, an adjuvant is added to the polysaccharide-CRM_{197} conjugate mixture, such as aluminum phosphate to 1 mg/mL final concentration. A calculated volume of sterile saline is added to reach the appropriate final concentration, and then the mixture is blended for approximately two hours. The formulate in the bulk container is then attached to the filling machine, which fills, stoppers, and seals the vials of the final vaccine product. These vial then are inspected, labeled, and packed for distribution. During this process, the pH, appearance of the vaccine, and the vaccine sterility is monitored. At the filling, stoppering, and sealing step, the appearance, pH, identity of polysaccharides, identity of CRM_{197}, sterility, saccharide content, total protein content, endotoxin content, pyrogen content, general safety, osmolality, and extractable volume are assessed. 1-5 microgram of polysaccharide per each serotype CRM_{197} conjugate is contained in a 0.5 mL of vaccine in a vial.

EXAMPLE 9

Rabbit Toxicology Safety Study

[0107] This example shows the rabbit toxicology studies that are used to assess vaccine safety. The polysaccharide polypeptide conjugate is produced according to the above **EXAMPLES 1**, **2**, **3**, and **5**, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide-CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly on days 1, 22, 43, 64, and 85 to rabbits, 5 months of age, (10/gender) at a dose of 0.5 ml/injection into the right thigh muscle. 0.5 ml 0.9% sodium chloride is injected in the Control groups (10/gender). 0.125 mg aluminum phosphate as an adjuvant is injected in the AlP04 group (10/gender). A 4 week dose free recovery period is undertaken in five (5) animals/gender/group after the last injection. Treatment-related mortality and any other toxicologically relevant changes in relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight is assessed to support lack of systemic toxicity. Injection site are

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analyzed for reactions and any chronic inflammation. Specific serum antibodies to each of the polysaccharides in the polysaccharide CRM_{197} conjugate vaccine is found in the test group.

[0108] The polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly with and without an aluminum phosphate adjuvant as a single 0.5 mL injection into the left thigh muscle of 3 male rabbits/group, 5 months of age. 0.5 ml 0.9% sodium chloride is administered to the control group (3 males). Treatment related mortality, changes in body weight, relative food consumption, or treatment related macroscopic or microscopic lesions is assessed.

EXAMPLE 10

Composition and Method of Administration of a Vaccine

[0109] This example shows a vaccine composition and a method of administration of that vaccine composition. An aqueous vaccine composition is composed of an immunogenic polysaccharide polypeptide conjugate comprising capsular polysaccharides from any serotype from **TABLE 1** conjugated to any one of diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof; or any combination of polysaccharide polypeptide conjugates as described in Example 6. The aqueous vaccine is formulated to contain from 1-4 microgram of each serotype of polysaccharide per polysaccharide polypeptide conjugate, aluminum phosphate (1 mg/mL), and 0.01% polysorbate 80 in a single 0.5 mL injection delivered intramuscularly to a subject. This vaccine is administered to subject, wherein the subject is a child of 4 weeks to 2 years old. This vaccine is administered to subject, wherein the subject is a child of 2 to 18 years

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old. This vaccine is administered to a subject, wherein the subject is an adult of at least 50 years of age.

EXAMPLE 11

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United States and Canada

[0110] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United States and/or Canada. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), such as serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United States and Canada is improved.

EXAMPLE 12

Vaccination for Protection against Endemic *S. pneumoniae* Serotypes in Europe, the Middle East, and North Africa

[0111] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Europe, the Middle East, and/or North Africa. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38, such as serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35F. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Europe, the Middle East, and North Africa is improved.

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EXAMPLE 13

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in Japan and Korea

[0112] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Japan and/or Korea. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38, such as serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Japan and Korea is improved.

EXAMPLE 14

Method of Making a 6C-CRM_{197} Conjugate Pharmaceutical Composition

[0113] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 6C serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 6C polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 6C polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 6C polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 6C-CRM_{197} conjugates. The 6C-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 6C-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 6C-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 15

Method of Making a 20B-CRM_{197} Conjugate Pharmaceutical Composition

[0114] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 20B serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 20B polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 20B

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polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 20B polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 20B-CRM_{197} conjugates. The 20B-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 20B-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 20B-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 16

Composition of a Kit with a Saccharide-Polypeptide Conjugate

[0115] This example shows the composition of a kit with a saccharide-polypeptide conjugate. A saccharide-polypeptide conjugate in a vial or a pre-filled syringe is placed in a container. The container is further associated with instructions of use.

EXAMPLE 17

Vaccination Against *S. pneumoniae*

[0116] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0117] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 18

Vaccination Against *S. pneumoniae*

[0118] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an

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immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0119] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 19

Vaccination Against *S. pneumoniae*

[0120] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes of any of the combinations listed in **TABLE 2**. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0121] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 20

Method of Vaccination

[0122] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an

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immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0123] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

EXAMPLE 21

Method of Vaccination

[0124] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0125] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

EXAMPLE 22

Method of Vaccination for an Adult Aged 50 Years Old or Older

[0126] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for an adult age 50 years old or older. A first aqueous vaccine composition is

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composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0127] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to an adult age 50 years old or older. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older.

EXAMPLE 23

Method of Vaccination for a Child Aged 2 to 18 Years Old

[0128] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 2 to 18 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0129] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 2 to 18 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old. Alternatively

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to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old.

EXAMPLE 24

Method of Vaccination for a Child Aged 4 Weeks to 2 Years Old

[0130] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 4 weeks to 2 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0131] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 4 weeks to 2 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child who is 2-18 years old. Alternatively to four weeks later, one year later a single 0.5 of the second aqueous vaccine is given by intramuscular injection to the child aged 4 weeks to 2 years old.

EXAMPLE 25

Method of Vaccination for *S. pneumoniae* and *N. meningitidis*

[0132] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *N. meningitidis*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C capsular saccharide conjugated to CRM_{197}. Each vaccine is formulated for intramuscular injection.

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[0133] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine is given by intramuscular injection.

EXAMPLE 26

Method of Vaccination for *S. pneumoniae* and *S. enterica*

[0134] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *S. enterica*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *S. enterica* subspecies enterica serovar Typhi Vi-capsular saccharide conjugated to CRM_{197}. Each aqueous vaccine is formulated for intramuscular injection.

[0135] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

EXAMPLE 27

Method of Vaccination for *S. pneumoniae*, *N. meningitidis,* and *H. influenzae*

[0136] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae*, *N. meningitidis,* and *H. influenzae*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to a tetanus toxoid carrier protein. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C and group Y saccharide and *H. influenzae* type b saccharide individually conjugated a tetanus toxoid carrier protein. Each aqueous vaccine is formulated for intramuscular injection.

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[0137] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

[0138] While preferred embodiments of the present invention have been shown and described herein, it will be apparent to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, and 38.
2. The pharmaceutical composition of claim 1, comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.
3. The pharmaceutical composition of claim 1, comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.
4. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
5. The pharmaceutical composition of claim 1, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a

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unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

6. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
7. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.
8. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
9. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
10. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
11. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype

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selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

12. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
13. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
14. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
15. The pharmaceutical composition of claim 1, comprising a plurality of at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B.
16. The pharmaceutical composition of claim 1, comprising a plurality of at least 8 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
17. The pharmaceutical composition of claim 1, comprising a plurality of at least 7 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular

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polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 15A, 15C, 16F, 23A, 23B, and 33B.

18. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
19. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
20. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
21. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.
22. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.

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23. The pharmaceutical composition of any one of claims 1-22, wherein the unique *Streptococcus pneumoniae* serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
24. A pharmaceutical composition comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 34, 35B, and 38.
25. The pharmaceutical composition of claim 24, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38.
26. The pharmaceutical composition of claim 24, comprising a plurality of at least 25 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.
27. The pharmaceutical composition of claim 24, comprising a plurality of at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B.
28. The pharmaceutical composition of claim 24, comprising a plurality of at least 19 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide,

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wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 24F, 35B and 38.

29. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.
30. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.
31. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
32. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B.
33. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
34. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.
35. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
36. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.

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37. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
38. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 23A, 23B, and 33B.
39. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
40. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
41. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
42. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
43. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
44. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
45. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.
46. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
47. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B.
48. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
49. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F.
50. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 20B, 23A, 23B, 33F, and 35B.

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51. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.
52. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 15A, 15C, 16F, 23A, 23B, and 33B.
53. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B.
54. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B, and 35B.
55. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31.
56. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B, 35F, and 35B.
57. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
58. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of the group consisting of 15A, 15C, 16F, 23A, 23B, and 33B.
59. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
60. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
61. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
62. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 20B and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.
63. The pharmaceutical composition of claim 62, wherein the at least one additional serotype consists of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B.

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64. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
65. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
66. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33B, 35F, and 35B.
67. A pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof conjugated to a polypeptide, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, and 38.
68. The pharmaceutical composition of claim 67, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.
69. The pharmaceutical composition of claim 67, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
70. The pharmaceutical composition of claim 67, further comprising at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38.
71. The pharmaceutical composition of any one of claims 1-70, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

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72. The pharmaceutical composition of any one of claims 1-71, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.
73. The pharmaceutical composition of claim 72, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.
74. The pharmaceutical composition of any one of claims 1-73, wherein at least one polypeptide of the plurality is an oligopeptide.
75. The pharmaceutical composition of any one of claims 1-74, wherein at least one polypeptide of the plurality comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.
76. The pharmaceutical composition of any one of claims 1-75, wherein the polypeptide comprises a mixture of polypeptides.
77. The pharmaceutical composition of any one of claims 1-76, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.
78. The pharmaceutical composition of any one of claims 1-77, further comprising an adjuvant.
79. The pharmaceutical composition in unit dose form of claim 78, wherein the adjuvant is an aluminum-cation comprising adjuvant.
80. The pharmaceutical composition of claim 78, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.
81. The pharmaceutical composition of any one of claims 1-80, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.
82. The pharmaceutical composition of any one of claims 1-81, further comprising a chelating agent.

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83. The pharmaceutical composition of claim 82, wherein the chelating agent comprises ethylenediaminetetraacetic acid (EDTA).
84. The pharmaceutical composition of any one of claims 1-83, which is in a form of a suspension.
85. The pharmaceutical composition of any one of claims 1-84, which is in the form of a homogenous mixture or a heterogeneous mixture.
86. The pharmaceutical composition of claim 85, wherein the pharmaceutical composition is in a form of a homogenous mixture.
87. The pharmaceutical composition of any one of claims 1-86, which is contained in a pre-filled syringe.
88. The pharmaceutical composition of any one of claims 1-87, further comprising a surfactant , an emulsifier, or a combination thereof.
89. The pharmaceutical composition of claim 88, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.
90. The pharmaceutical composition of claim 89, comprising the polysorbate, wherein the polysorbate is polysorbate 80.
91. The pharmaceutical composition of claim 89, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.
92. The pharmaceutical composition of any one of claims 1-91, further comprising a buffering agent.
93. The pharmaceutical composition of claim 92, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.
94. The pharmaceutical composition of any one of claims 1-93, further comprising sterile water.
95. The pharmaceutical composition of any one of claims 1-94, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.
96. The pharmaceutical composition of any one of claims 1-95, wherein when the pharmaceutical composition is administered intramuscularly to a human, the

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pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

97. The pharmaceutical composition of any one of claims 1-96, wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

98. The pharmaceutical composition of any one of claims 1-97, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

99. The pharmaceutical composition of any one of claims 1-98, wherein the pharmaceutical composition is in unit dose form.

100. The pharmaceutical composition of any one of claims 1-99, further comprising a preservative.

101. The pharmaceutical composition of any one of claims 1-100, further comprising an anti-fungal compound or a salt thereof.

102. The pharmaceutical composition of any one of claims 1-101, further comprising a salt.

103. The pharmaceutical composition of any one of claims 1-102, wherein the salt is an inorganic salt.

104. The pharmaceutical composition of any one of claims 1-103, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

105. The pharmaceutical composition of any one of claims 1-104, which is a vaccine.

106. The pharmaceutical composition of any one of claims 1-105, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

107. The pharmaceutical composition of any one of claims 1-106, wherein the immunogenic saccharide-polypeptide conjugate is produced using bacteria.

108. The pharmaceutical composition of any one of claims 1-107, wherein the immunogenic saccharide-polypeptide conjugate is produced using *E. coli*.

109. The pharmaceutical composition of any one of claims 1-108, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

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110. The pharmaceutical composition of any one of claims 1-109, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

111. The pharmaceutical composition of any one of claims 1-110, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

112. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 1-111.

113. The method of claim 112, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising an immunogenic saccharide-polypeptide conjugate comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

114. The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

115. The method of any one of claims 113-114, wherein the second composition comprises a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

116. The method of any one of claims 113-115, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

117. The method of any one claims 113-116, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising each

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comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.

118. The method of claim 117, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.

119. The method of claim 118, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a fragment of the capsular polysaccharide.

120. The method of claim 119, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

121. The method of any one of claims 113-120, wherein the second polypeptide is an oligopeptide.

122. The method of any one of claims 113-121, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.

123. The method of any one of claims 113-122, wherein a toxin activity of the second polypeptide is at least partly mitigated.

124. The method of any one of claims 113-123, wherein the second composition further comprises an adjuvant.

125. The method of claim 124, wherein the adjuvant is an aluminum-cation comprising adjuvant.

126. The method of claim 125, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

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127. The method any one of claims 113-126, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

128. The method of any one of claims 113-127, wherein the second composition is in unit dose form.

129. The method of any one of claims 113-128, wherein the second composition further comprises a chelating agent.

130. The method of claim 129, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

131. The method any one of claims 113-130, wherein the second composition is in a form of a suspension.

132. The method any one of claims 113-131, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.

133. The method any one of claims 113-132, wherein the second composition is in the form of a homogenous mixture.

134. The method any one of claims 113-133, wherein the second composition is contained in a pre-filled syringe.

135. The method any one of claims 113-134, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.

136. The method claim 135, wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

137. The method of claim 136, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.

138. The method of claim 137, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

139. The method any one of claims 113-138, wherein the second composition further comprises a buffering agent.

140. The method of claim 139, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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141. The method any one of claims 113-140, wherein the second composition further comprises sterile water.

142. The method any one of claims 113-141, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

143. The method any one of claims 113-142, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

144. The method any one of claims 113-143, wherein when the unique immunogenic saccharide-polypeptide conjugate of the second composition elicits an opsonophagocytic response.

145. The method of any one of claims 113-144, wherein when the second composition is administered intramuscularly, the second composition elicits an immune response that lasts from about 1 week to about 30 years.

146. The method of any one of claims 113-145, wherein second composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

147. The method of any one of claims 113-146, wherein the second composition further comprises a preservative.

148. The method of any one of claims 113-147, wherein the second composition further comprises an anti-fungal compound or a salt thereof.

149. The method of any one of claims 113-148, wherein the second composition further comprises a salt.

150. The method of claims 149, wherein the salt is an inorganic salt.

151. The method of any one of claims 113-150, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is isolated and purified.

152. The method of any one of claims 113-151, wherein the second composition is a vaccine.

153. The method of any one of claims 113-152, which is a method of vaccinating a subject.

154. The method of claim 153, wherein the subject is a human.

155. The method of any one of claims 153-154, wherein the subject or human is a subject or human in need thereof.

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156. The method of any one of claims 113-155, wherein the immunogenic saccharide-polypeptide conjugates of the second composition are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

157. The method of any one of claims 113-156, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate of the second composition, wherein the antibody response is measured by measuring antibody titers.

158. The method of any one of claims 113-157, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using bacteria.

159. The method of any one of claims 113-158, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using *E. coli*.

160. The method of any one of claims 113-159, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

161. The method of any one of claims 113-160, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

162. The method of any one of claims 113-161, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

163. A method comprising:

administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition;

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and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

164. The method of claim 163, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

165. The method of claim 164, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

166. The method of claim 165, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

167. The method of any one of claims 163-166, wherein the first polypeptide is an oligopeptide.

168. The method of any one of claims 163-167, wherein the second polypeptide is an oligopeptide.

169. The method of any one of claims 163-168, wherein the first polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof .

170. The method of any one of claims 163-169, wherein a toxin activity of the first polypeptide is at least partly mitigated.

171. The method of any one of claims 163-170, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.

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172. The method of any one of claims 163-171, wherein a toxin activity of the second polypeptide is at least partly mitigated.

173. The method of any one of claims 163-172, wherein the first serotype is from a same bacteria as the second serotype.

174. The method of any one of claims 163-173, wherein the first serotype is from a different bacteria than the second serotype.

175. The method of any one of claims 163-174, wherein the first serotype is from *Streptococcus pneumoniae*.

176. The method of any one of claims 163-174, wherein the first serotype is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

177. The method of any one of claims 163-176, wherein the second serotype is from *Streptococcus pneumoniae*.

178. The method of any one of claims 163-176, wherein the second bacteria is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

179. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

180. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

181. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

182. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

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183. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*.

184. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*.

185. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*.

186. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*.

187. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*.

188. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

189. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 15A, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

190. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

191. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus*

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pneumoniae, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

192. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

193. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

194. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

195. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

196. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

197. The method of any one of claims 163-196, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

198. The method of claim 197, wherein the adjuvant is an aluminum-cation comprising adjuvant.

199. The method of claim 198, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

200. The method of any one of claims 163-199, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable

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composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

201. The method of any one of claims 163-200, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

202. The method of claim 201, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

203. The method of any one of claims 163-202, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

204. The method of any one of claims 163-203, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

205. The method of any one of claims 163-204, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

206. The method of any one of claims 163-205, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

207. The method of any one of claims 163-206, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

208. The method of claim 207, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

209. The method of claim 208, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

210. The method claim 208, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

211. The method of any one of claims 163-210, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

212. The method of claim 211, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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213. The method of any one of claims 163-212, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

214. The method of any one of claims 163-213, wherein when the first immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

215. The method of any one of claims 163-214, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

216. The method any one of claims 163-215, wherein when first immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

217. The method of any one of claims 163-216, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

218. The method of any one of claims 163-217, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

219. The method of claim 218, wherein the adjuvant is an aluminum-cation comprising adjuvant.

220. The method of claim 218, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

221. The method of any one of claims 163-220, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

222. The method of any one of claims 163-221, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

223. The method of claim 222, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

224. The method of any one of claims 163-223, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

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225. The method of any one of claims 163-224, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

226. The method of claim 225, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

227. The method of any one of claims 163-226, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

228. The method of any one of claims 163-227, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

229. The method of claim 228, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

230. The method of claim 229, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

231. The method of claim 230, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

232. The method of any one of claims 163-231, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

233. The method of claim 232, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

234. The method of any one of claims 163-233, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

235. The method of any one of claims 163-234, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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236. The method of any one of claims 163-235, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

237. The method of any one of claims 163-236, wherein when the second immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

238. The method of any one of claims 163-237, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

239. The method of any one of claims 163-238, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is in unit dose form.

240. The method of any one of claims 163-239, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises a preservative.

241. The method of any one of claims 163-240, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises an anti-fungal compound or a salt thereof.

242. The method of any one of claims 163-241, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprise a salt.

243. The method of claim 242, wherein the salt is an inorganic salt.

244. The method of any one of claims 163-243, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is isolated and purified.

245. The method of any one of claims 163-244, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition which is a vaccine.

246. The method of any one of claims 163-245, which is a method of vaccinating a subject.

247. The method of any claim 246, wherein the subject is a human.

248. The method of any one of claims 246-247, wherein the subject or human is a subject or human in need thereof.

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249. The method of any one of claims 163-248, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprise immunogenic saccharide-polypeptide conjugate(s) individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

250. The method of any one of claims 163-249, wherein administration of the second immunogenic saccharide-polypeptide conjugate composition does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

251. The method of any one of claims 163-250, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using a bacteria.

252. The method of any one of claims 163-251, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using *E. coli*.

253. The method of any one of claims 163-252, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

254. The method of any one of claims 163-253, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

255. The method of any one of claims 163-254, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

256. A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 1-70 with an excipient, an adjuvant, or any combination thereof.

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257. The method of claim 256, wherein the composition is injectable or intranasally administrable.

258. The method claim 256, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

259. The method of any one of claims 256-258, wherein the composition is in the form of an intramuscularly injectable composition.

260. The method of any one of claims 256-259, further comprising contacting the composition with a chelating agent.

261. The method of claim 260, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

262. The method of any one of claims 256-261, wherein the composition is in a form of a suspension.

263. The method of any one of claims 256-262, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

264. The method of any one of claims 263, wherein the composition is in a form of a homogenous mixture.

265. The method of any one of claims 256-264, wherein the composition is contained in a pre-filled syringe.

266. The method of any one of claims 256-265, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

267. The method of claim 266, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

268. The method of claim 267, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

269. The method of claim 268, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

270. The method of any one of claims 256-269, further comprising contacting the composition with a buffering agent.

271. The method of claim 270, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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272. The method of any one of claims 256-271, further comprising contacting the composition with sterile water.

273. The method of any one of claims 256-272, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

274. The method of any one of claims 256-273, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

275. The method of any one of claims 256-274, wherein the immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

276. The method of any one of claims 256-275, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

277. The method of any one of claims 256-276, wherein the composition further comprises a preservative.

278. The method of any one of claims 256-277, wherein the composition further comprises an anti-fungal compound or a salt thereof.

279. The method of any one of claims 256-278, wherein the composition further comprises a salt.

280. The method claim 279, wherein the salt is an inorganic salt.

281. The method of any one of claims 256-280, wherein the immunogenic saccharide-polypeptide conjugate of the composition is isolated and purified.

282. The method of any one of claims 256-281, wherein the composition is used as a vaccine.

283. The method of any claims 256-282, wherein the composition is used for vaccinating a subject.

284. The method of claim 283, wherein the subject is a human.

285. The method of any one of claims 267-284, wherein the subject or human is a subject or human in need thereof.

286. The method of any one of claims 256-285, wherein the immunogenic saccharide-polypeptide conjugate(s) of the composition are individually, respectively, or collectively

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present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

287. The method of any one of claims 256-286, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using bacteria.

288. The method of any one of claims 256-287, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using E. coli.

289. The method of any one of claims 256-288, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

290. The method of any one of claims 256-289, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

291. The method of any one of claims 256-290, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

292. A kit comprising of a pharmaceutical composition of any of claims 1-111 contained in a container.

293. The kit of claim 292, further comprising instructions for use.

294. The kit of any one of claims 292-293, wherein the container is a syringe.

295. The kit of any one of claims 292-294, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

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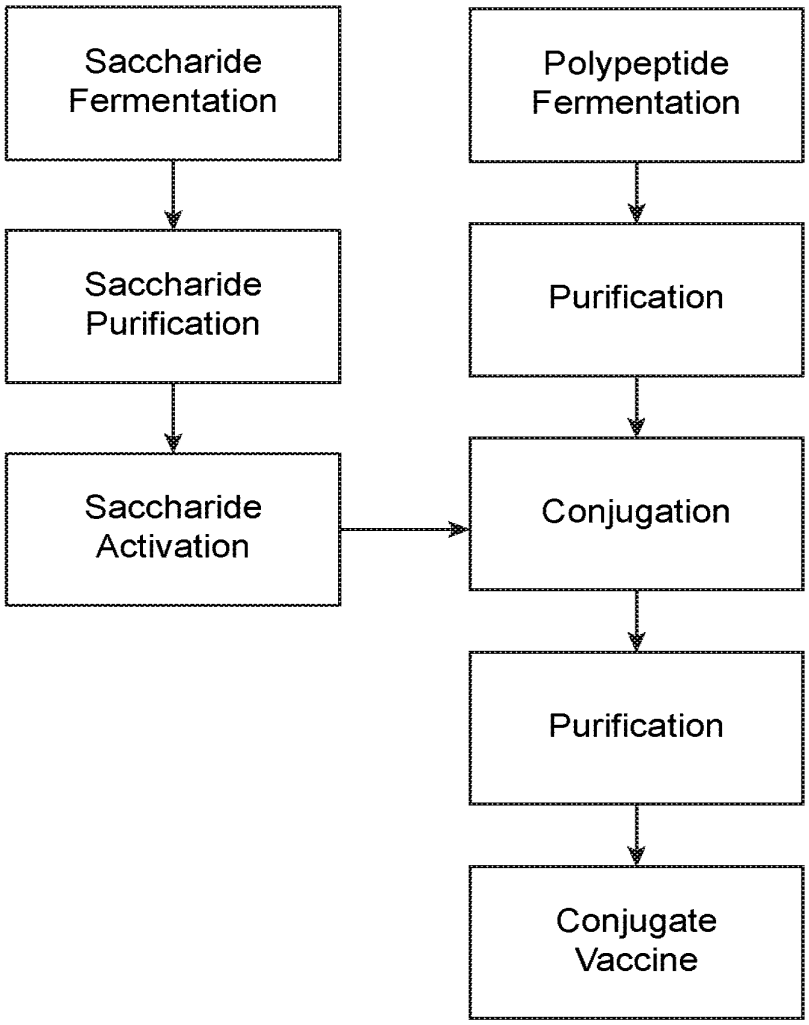


FIG. 1

Petitioner Merck Ex-1004, 0118

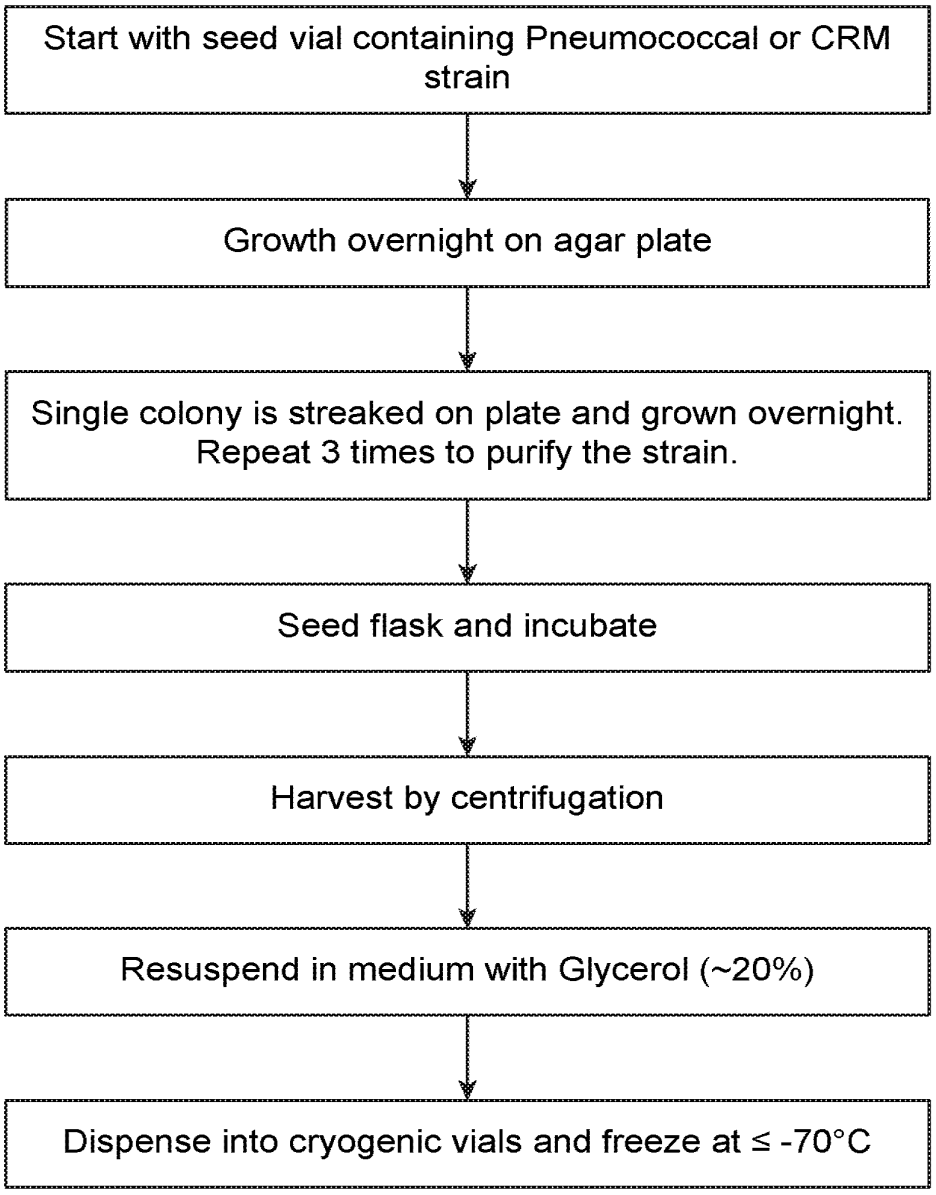


FIG. 2

Petitioner Merck Ex-1004, 0119

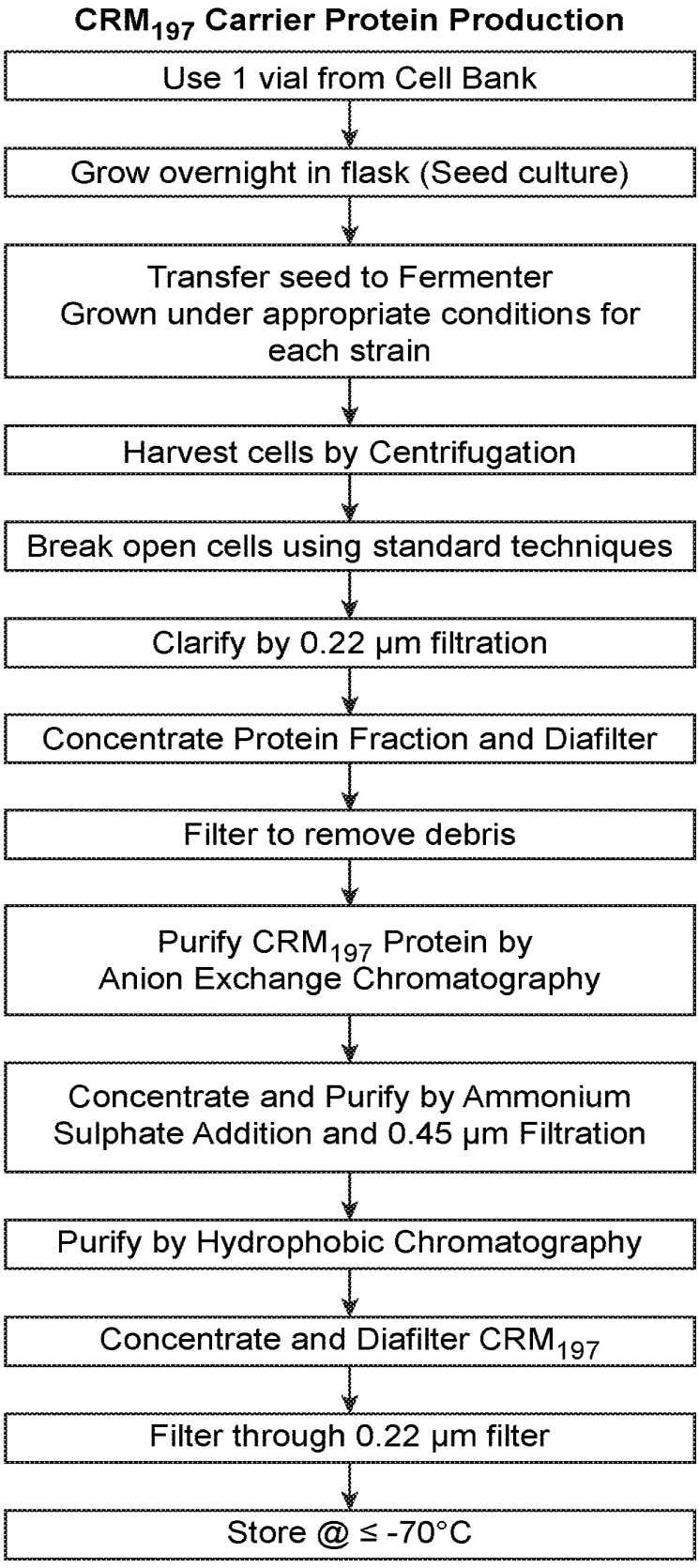


FIG. 3

Petitioner Merck Ex-1004, 0120

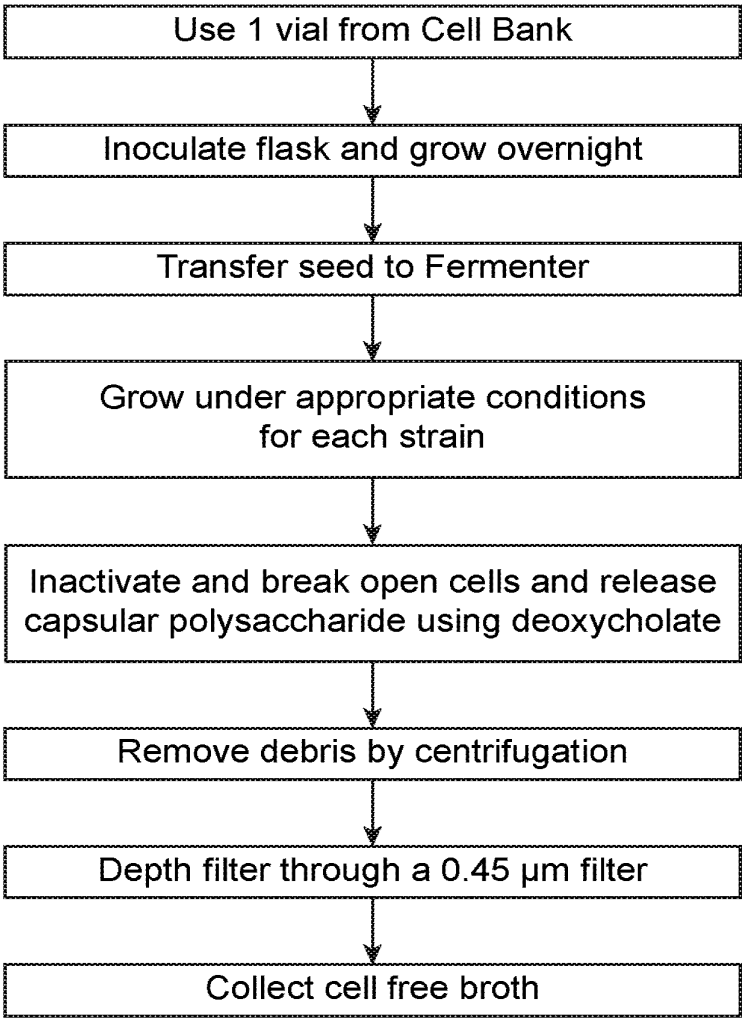


FIG. 4

Petitioner Merck Ex-1004, 0121

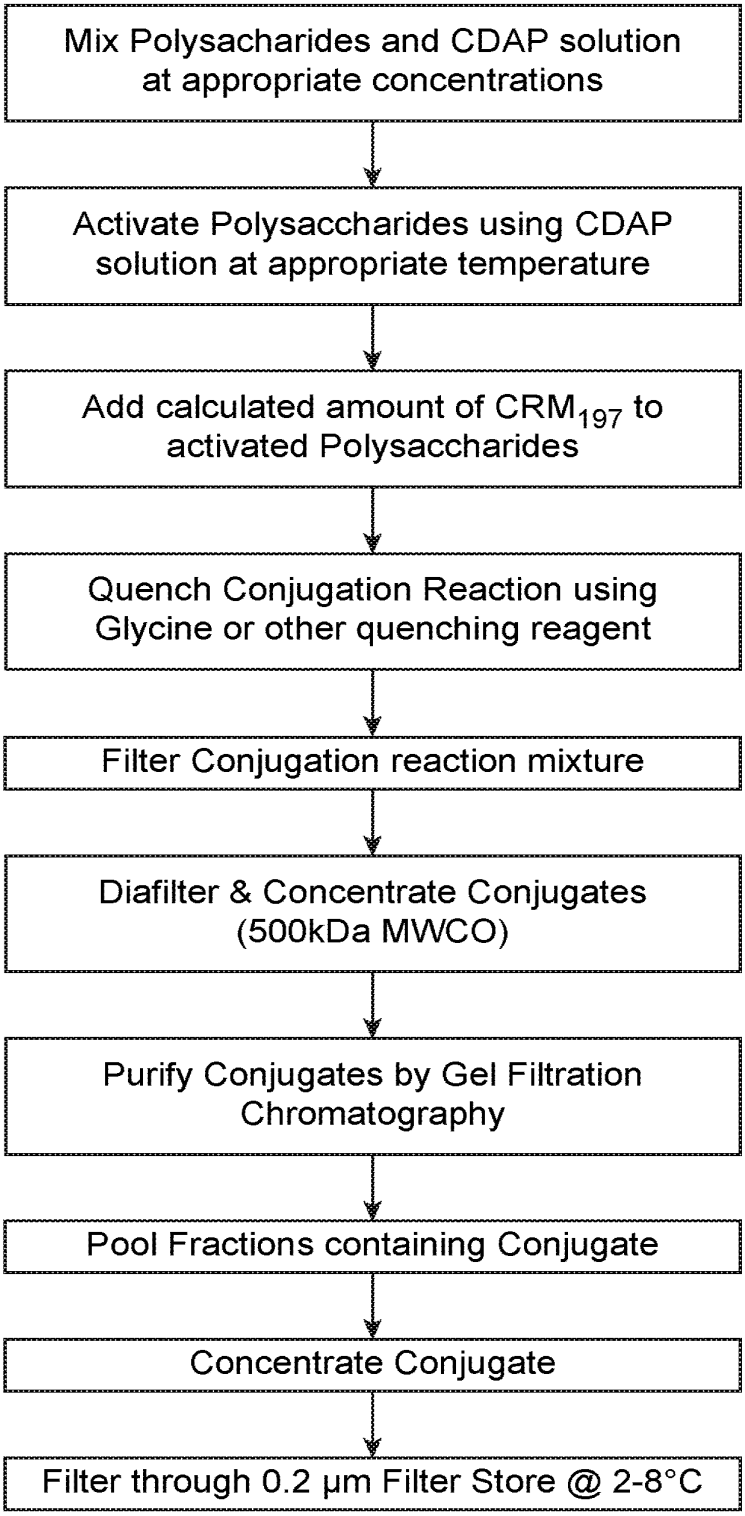


FIG. 5

Petitioner Merck Ex-1004, 0122

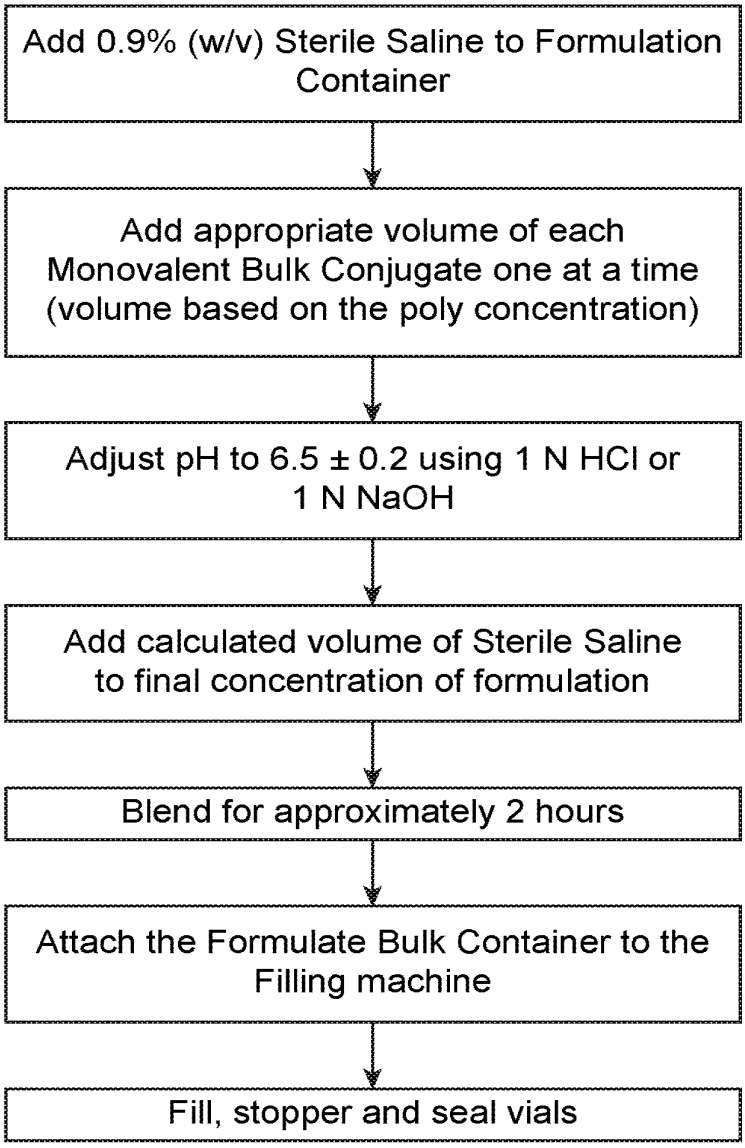


FIG. 6

Petitioner Merck Ex-1004, 0123

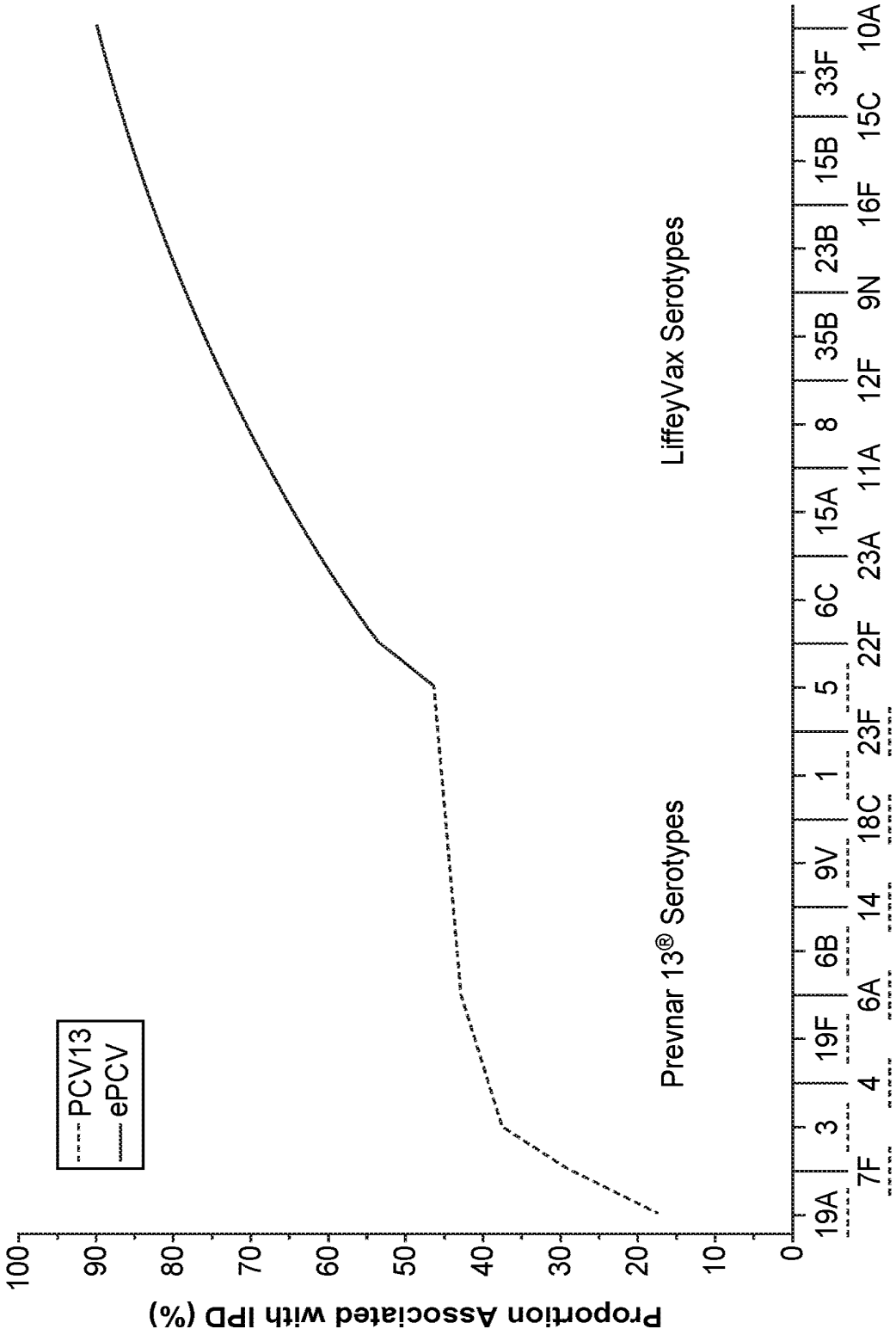


FIG. 7

Petitioner Merck Ex-1004, 0124

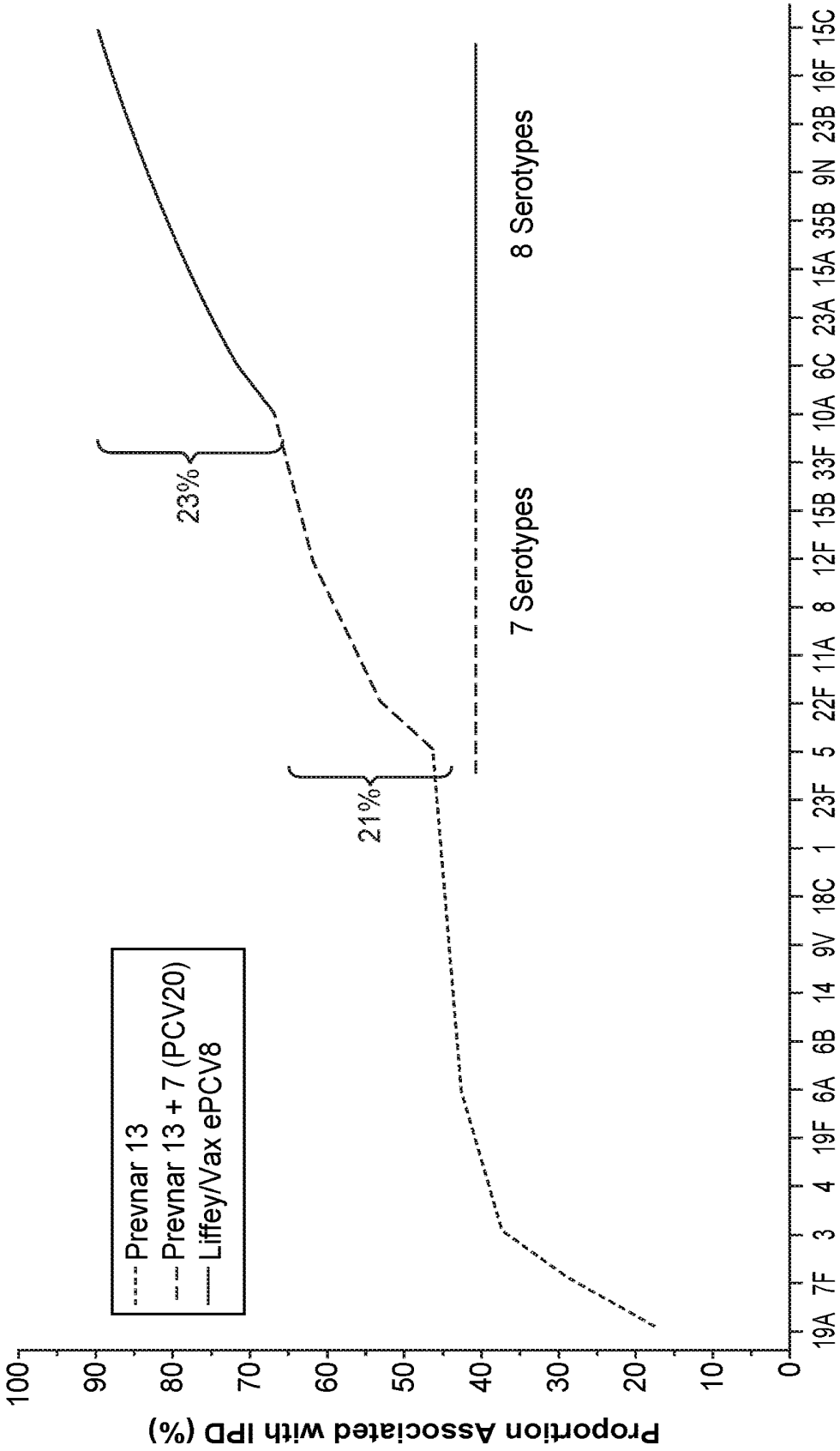


FIG. 8

Petitioner Merck Ex-1004, 0125

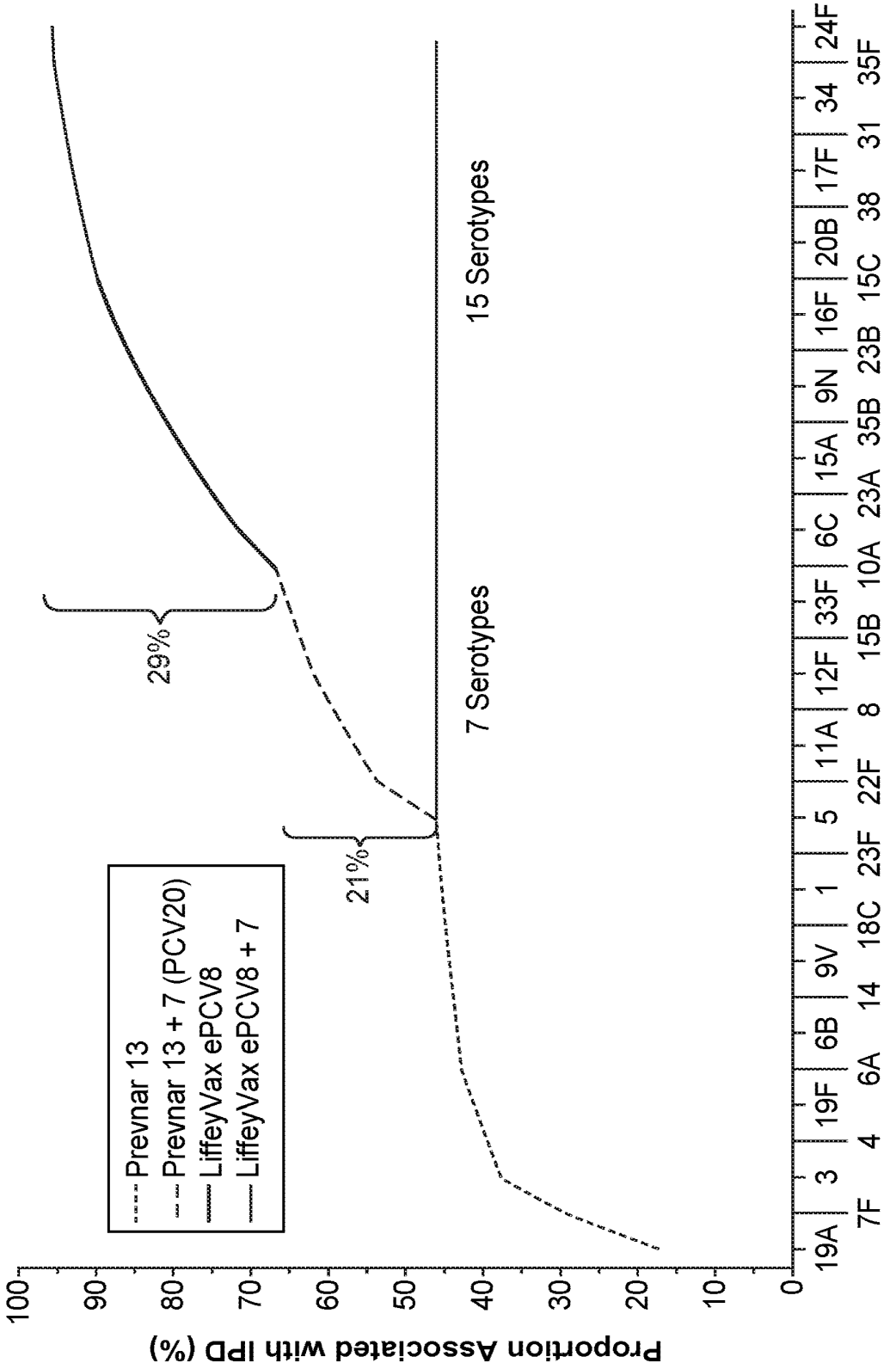


FIG. 9

Petitioner Merck Ex-1004, 0126

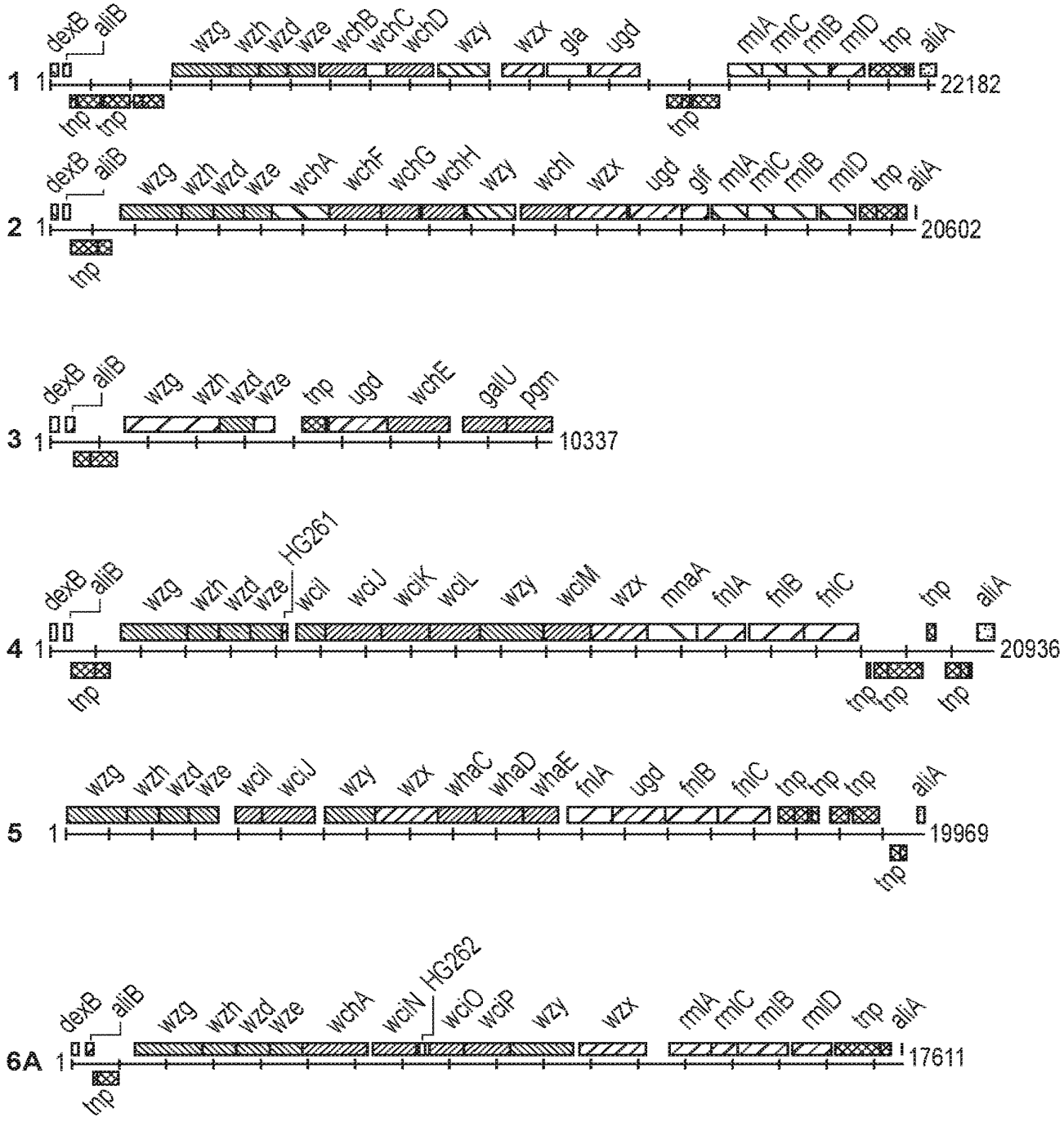


FIG. 10A

Petitioner Merck Ex-1004, 0127

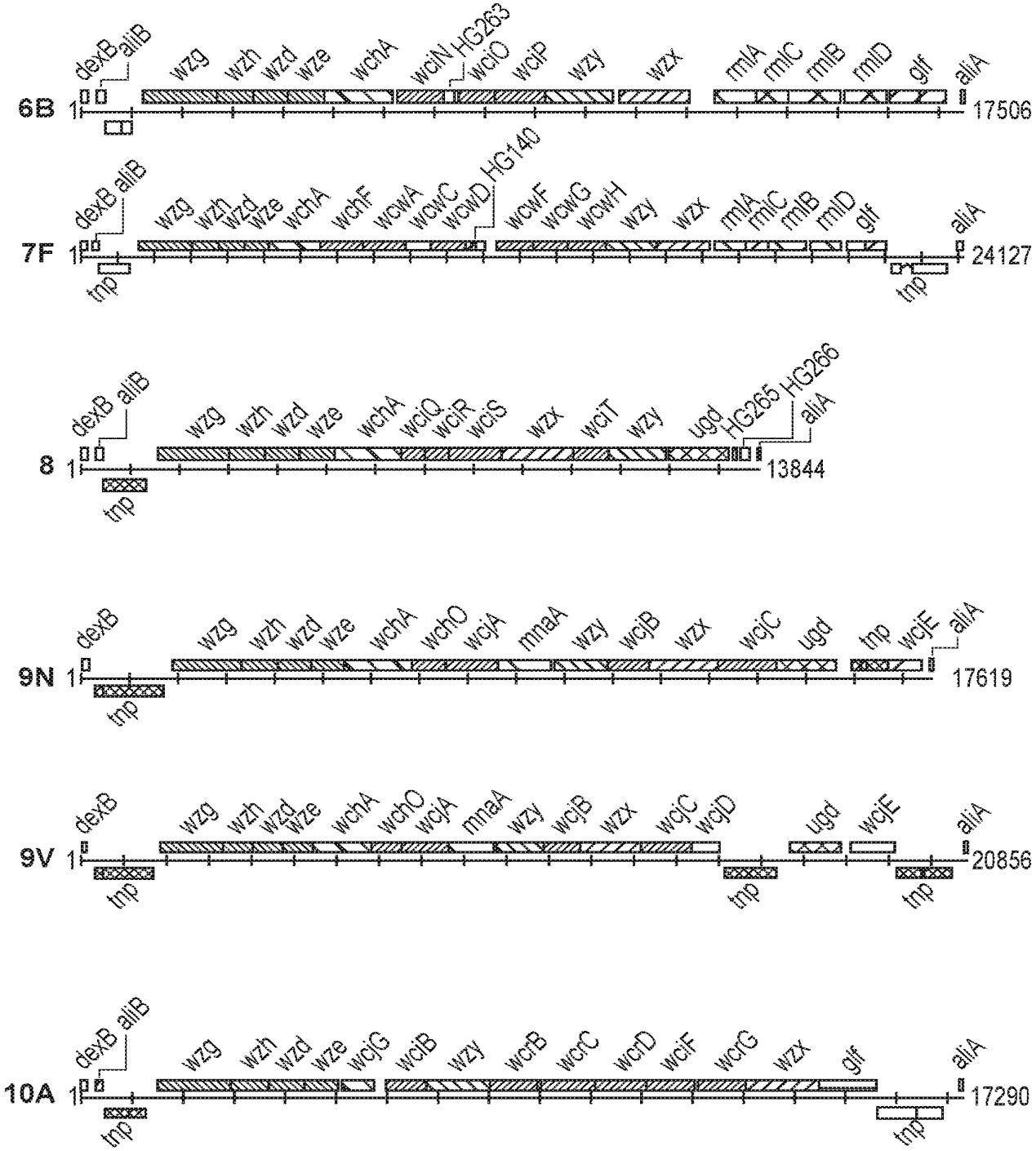


FIG. 10B

Petitioner Merck Ex-1004, 0128

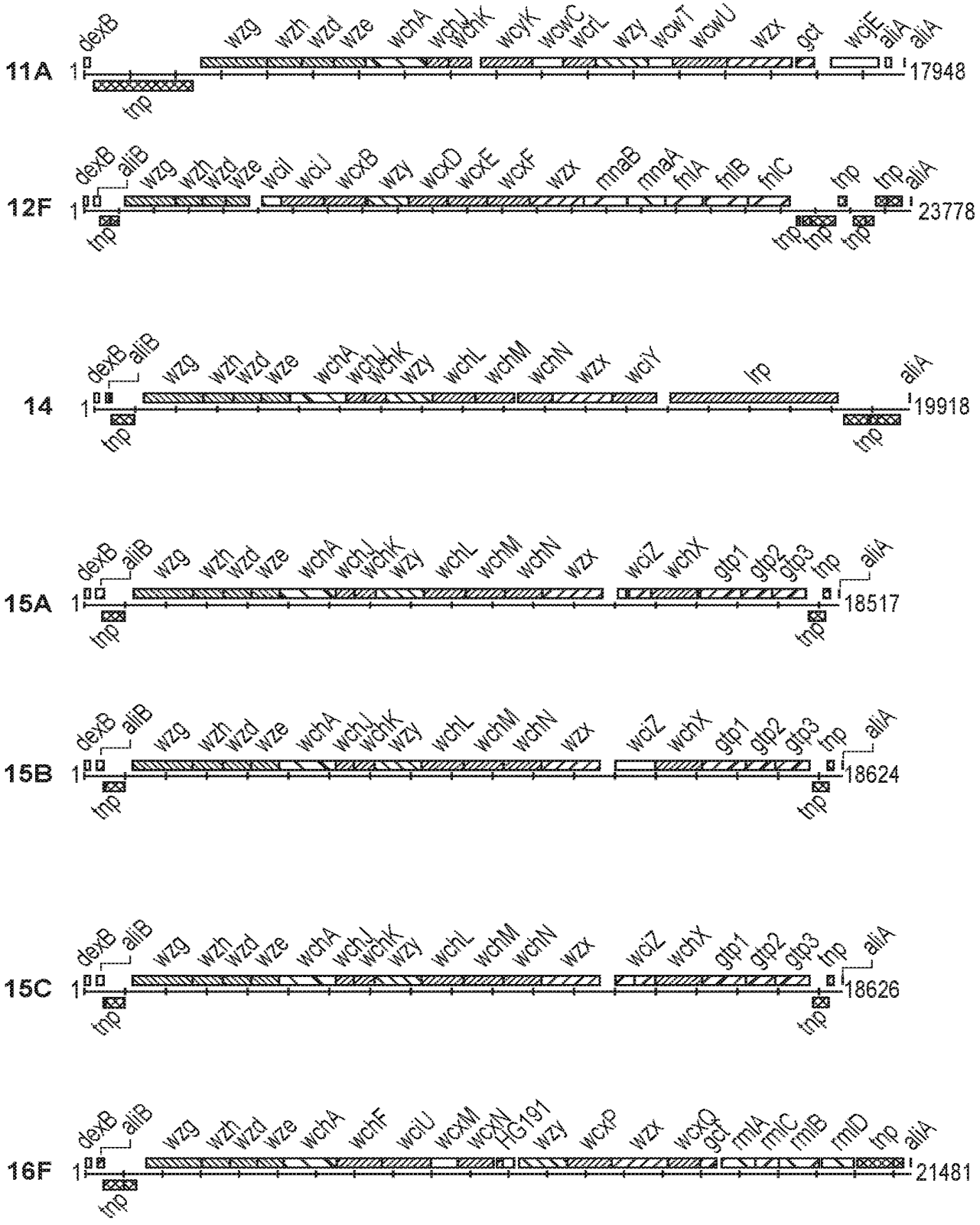


FIG. 10C

Petitioner Merck Ex-1004, 0129

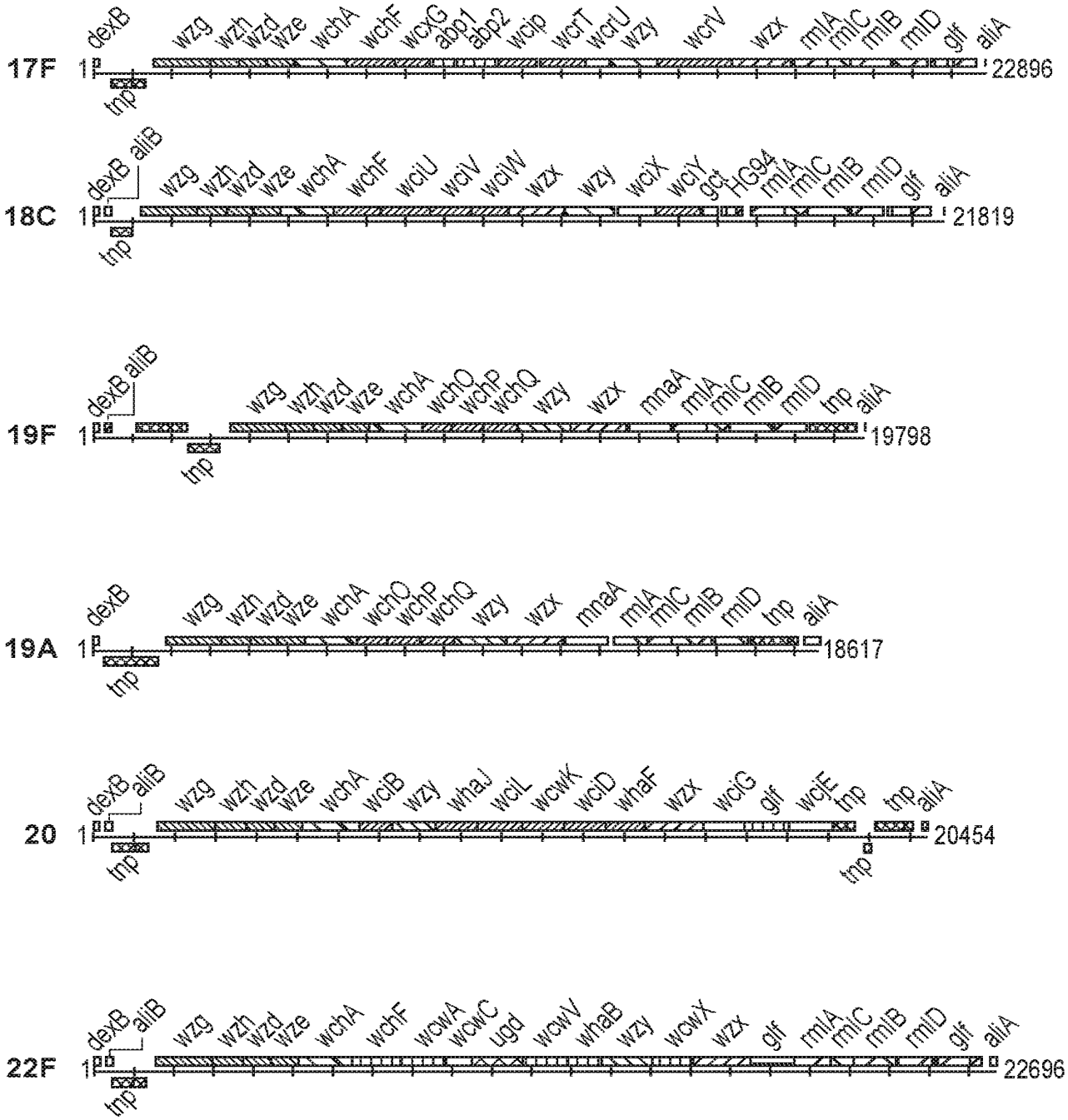


FIG. 10D

Petitioner Merck Ex-1004, 0130

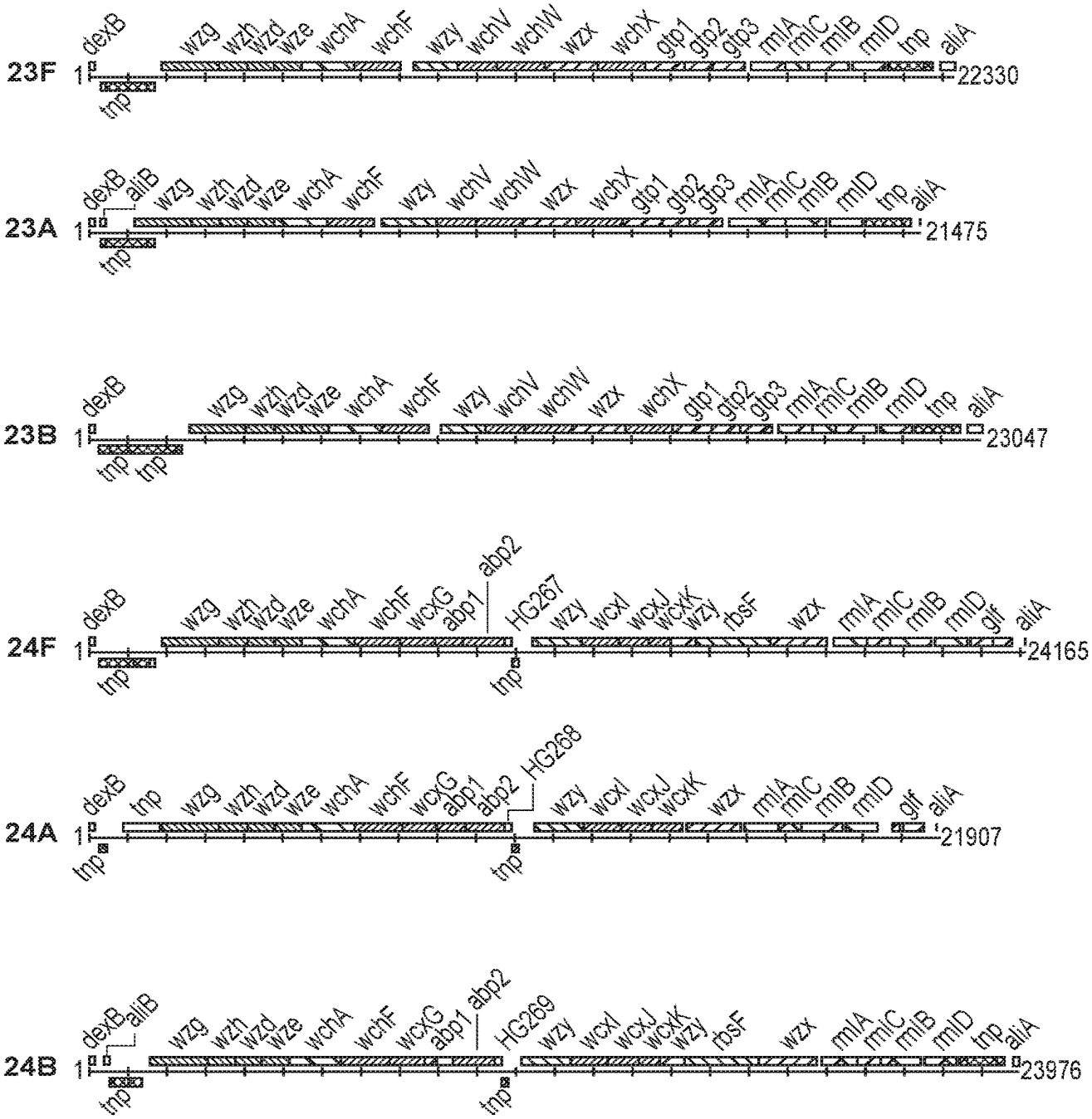


FIG. 10E

Petitioner Merck Ex-1004, 0131

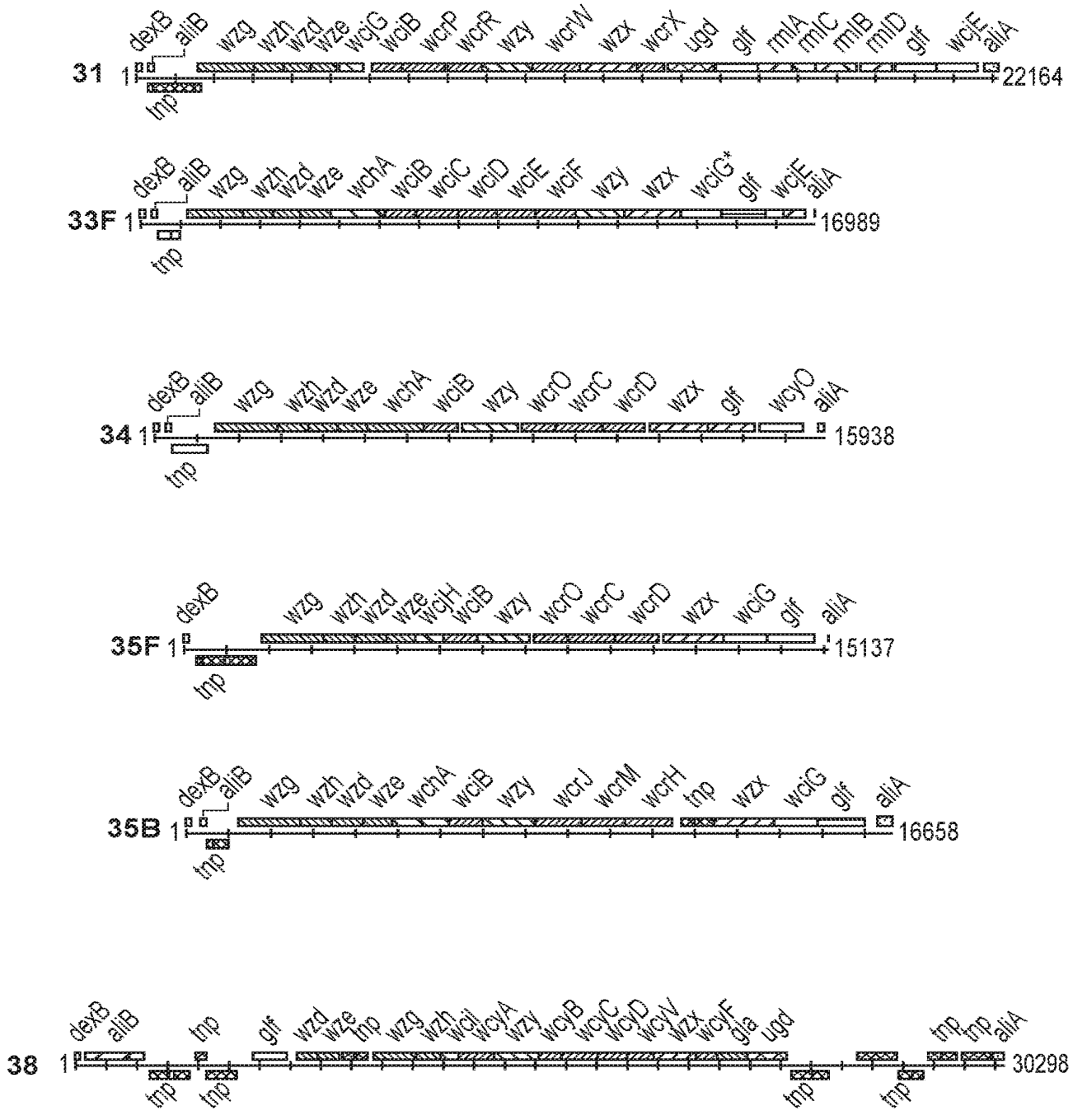


FIG. 10F

Petitioner Merck Ex-1004, 0132

PTO-1390 (01-18)
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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371

Attorney Docket No.	
50308-701.831	
U.S. Application No. (if known, see 37 CFR 1.5)	

International Application No.	International Filing Date	Priority Date Claimed
PCT/US2017/025621	03-31-2017	03-31-2016

Title of Invention
SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

First Named Inventor
Bruce D. FORREST

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received.
2. ☐ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)).
3. An English language translation of the International Application (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
   a. ☐ is attached.
   b. ☐ was previously filed in the international phase under PCT Rule 4.17(iv).

Items 5 to 8 below concern amendments made in the international phase.

PCT Article 19 and 34 amendments

5. ☐ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)).
6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)).
7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)).

Cancellation of amendments made in the international phase

8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19.

8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34.

NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s).

The following items 9 to 17 concern a document(s) or information included.

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
10. ☐ A preliminary amendment.
11. ■ An Application Data Sheet under 37 CFR 1.76.
12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b).
13. ☐ A power of attorney and/or change of address letter.
14. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821-1.825 (not required if sequence listing in text format was indicated on the PCT Request as part of the International Application and the sequence listing was published as part of the international application).
15. ☐ Assignment papers *(cover sheet and document(s))*. Name of Assignee: _____
16. ☐ 37 CFR 3.73(c) Statement *(when there is an Assignee)*.

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 15 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

U.S. APPLN. No. (if known – see 37 CFR 1.5)	INTERNATIONAL APPLICATION No.	ATTORNEY DOCKET No.
	PCT/US2017/025621	50308-701.831

17. ☐ Other items or information:

The following fees have been submitted.	CALCULATIONS
18. ■ Basic national fee (37 CFR 1.492(a)) **$300**	$ 300
19. ■ Examination fee (37 CFR 1.492(c)) • If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0** • All other situations **$760**	$ 760
20. ■ Search fee (37 CFR 1.492(b)) • If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0** • Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority .....**$140** • International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB.....**$520** • All other situations **$660**	140 $
TOTAL OF 18, 19, and 20 =	$ 1200
☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). Fee for each additional 50 sheets of paper or fraction thereof **$400**	

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round **up** to a whole number)	RATE	
- 100 =	/ 50 =		x **$400**	$

Surcharge for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h))..... **$140**	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =		x **$100**	$
Independent claims	- 3 =		x **$460**	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$820**	$

Fee for submission of Sequence Listing text file of 300 MB to 800 MB (37 CFR 1.21(o)(1))..... **$1,000**	$
Fee for submission of Sequence Listing text file of more than 800 MB (37 CFR 1.21(o)(2))..... **$10,000**	$
Processing fee for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i))..... **$140** +	$
TOTAL OF ABOVE CALCULATIONS =	$ 1200
■ **Applicant asserts small entity status.** See 37 CFR 1.27. Fees above are reduced by ½.	
☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Fees above are reduced by ¾. Applicant must attach form PTO/SB/15A or B or equivalent.	
TOTAL NATIONAL FEE =	$ 600
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31)..... **$50.00** per property +	$
TOTAL FEES ENCLOSED =	$ 600

Petitioner Merck Ex-1004, 0134

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 232415 in the amount of $ 600 to cover the above fees.

c. ☐ The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. _____ as follows:

i. ☐ any required fee.

ii. ☐ any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended to pay fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA**.

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

☒ The address associated with Customer Number: 021971 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone			
Email					

Signature	/Keli Holzapfel/	Date	09-27-2018
Name (Print/Type)	Keli Holzapfel	Registration No. (Attorney/Agent)	65,254

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Filer:	Keli Holzapfel/Cybele Radcliff
Attorney Docket Number:	50308-701.831

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
BASIC NATIONAL STAGE FEE	2631	1	150	150
NATL STAGE SEARCH FEE - U.S. WAS THE ISA	2641	1	70	70
NATL STAGE EXAM FEE - ALL OTHER CASES	2633	1	380	380
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**600**

Electronic Acknowledgement Receipt

EFS ID:	33848172
Application Number:	16089040
International Application Number:	PCT/US2017/025621
Confirmation Number:	8702
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Customer Number:	21971
Filer:	Keli Holzapfel/Cybele Radcliff
Filer Authorized By:	Keli Holzapfel
Attorney Docket Number:	50308-701.831
Receipt Date:	27-SEP-2018
Filing Date:	
Time Stamp:	14:36:38
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$600
RAM confirmation Number	092818INTEFSW00001185232415
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	50308_701_831_ADS.pdf	178389 06bf9e35423801 0f50bbd2b6d59461956b28430d	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2	Transmittal of New Application	50308_701_831_Transmittal.pdf	197653 f2bfece0f33560cf014125dd2d9e6d34480b03e5	no	4
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	35488 7e33ebdd5524d862dc0d932078878c3c30f1f453	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	411530		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Bruce	D.	Forrest	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	South Nyack	State/Province	NY	Country of Residence	US

Mailing Address of Inventor:

Address 1	135 Piermont Avenue		
Address 2			
City	South Nyack	State/Province	NY
Postal Code	10960	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jack	D.	Love	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Tampa	State/Province	FL	Country of Residence	US

Mailing Address of Inventor:

Address 1	4111 W. Inman Avenue		
Address 2			
City	Tampa	State/Province	FL
Postal Code	33609	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	021971		
Email Address	patentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF		
Attorney Docket Number	50308-701.831	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	15	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	021971		

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	a 371 of international	PCT/US2017/025621	2017-03-31
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2017/025621	Claims benefit of provisional	62316555	2016-03-31
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2017/025621	Claims benefit of provisional	62330245	2016-05-02
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2017/025621	Claims benefit of provisional	62372263	2016-08-08
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2017/025621	Claims benefit of provisional	62373807	2016-08-11

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◯ Assignee	◯ Legal Representative under 35 U.S.C. 117	◯ Joint Inventor
◯ Person to whom the inventor is obligated to assign.	◯ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Liffey Biotech Limited		
Mailing Address Information For Applicant:			
Address 1	c/o Mathesons		
Address 2	70 Sir John Rogersons Quay		
City	Dublin	**State/Province**	IE
Country	IE	Postal Code	D02 R296
Phone Number		Fax Number	
Email Address	patentdocket@wsgr.com		

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Keli Holzapfel/			Date (YYYY-MM-DD)	2018-09-27
First Name	Keli	Last Name	Holzapfel	Registration Number	65,254

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.831
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Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

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SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. provisional patent application number 62/316,555, filed March 31, 2016; U.S. provisional patent application number 62/330,245, filed May 2, 2016; U.S. provisional patent application number 62/372,263, filed August 8, 2016; and U.S. provisional patent application number 62/373,807, filed August 11, 2016, each of which are herein incorporated by reference in their entirety.

SUMMARY

[0002] Provided herein is a brief summary. This summary can be used to provide an understanding of embodiments provided herein, but the embodiments are not limited to what is provided in this summary.

[0003] Provided herein is a pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 6C and at least one additional serotype

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is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition, which can be in unit dose form, comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 20B and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

[0004] A pharmaceutical composition, which can be in unit dose form, comprising an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof conjugated to a polypeptide, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The pharmaceutical composition can further comprise at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B).

[0005] Provided herein is a method comprising administering to a subject a first composition, wherein the first composition is one of any pharmaceutical composition described herein. Further provided herein is a method comprising administering a first composition and a second composition to a subject, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising an immunogenic saccharide-polypeptide conjugate comprising a second polypeptide to the subject, wherein the second polypeptide

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has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

[0006] Provided herein is a method comprising administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

[0007] Further provided herein is a method of making a composition comprising contacting an immunogenic saccharide-polypeptide conjugate of any one of the described pharmaceutical compositions with an excipient, an adjuvant, or any combination thereof.

[0008] Provided herein is a kit comprising of any pharmaceutical composition described herein or any pharmaceutical composition contained in a container described herein.

[0009] Provided herein are methods of making compositions, methods of immunizing with compositions, and methods of vaccinating with compositions.

INCORPORATION BY REFERENCE

[0010] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0011] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0012] FIGURE 1 illustrates a flow diagram of the general process for manufacture of a saccharide peptide conjugate vaccine.

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[0013] FIGURE 2 illustrates a flow diagram of a process for cell banking of a bacterial strain.

[0014] FIGURE 3 illustrates a flow diagram of a process for producing, isolating, and purifying CRM_{197}.

[0015] FIGURE 4 illustrates a flow diagram of a fermentation process for isolating a polysaccharide from a strain of *Streptococcus pneumoniae*.

[0016] FIGURE 5 illustrates a flow diagram of a conjugation process for conjugating a polysaccharide and CRM_{197}.

[0017] FIGURE 6 illustrates a flow diagram for a process for formulating a polysaccharide polypeptide conjugate vaccine.

[0018] FIGURE 7 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B (ePCV; LiffeyVax Serotypes) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13). Prevnar 13 alone only covers about 46.1% of IPD compared to an overall coverage of 90% when administered in a vaccination regime with ePCV.

[0019] FIGURE 8 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B (Liffey/Vax ePCV8) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of the eight serotypes in the Liffey/Vax ePCV8 is predicted to cover about 23% more of IPD when administered in a vaccination regime with PCV20.

[0020] FIGURE 9 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38 (ePCV8+7) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven

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serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of seven serotypes in the Liffey/Vax ePCV8 (Liffey/Vax ePCV8+7) (see **FIGURE 8** for ePCV8) is predicted to cover about 29% more of IPD when administered in a vaccination regime with PCV20.

[0021] FIGURES 10A, 10B, 10C, 10D, 10E, and 10F illustrate annotated the capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley et al., Genetic Anaylsis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

DETAILED DESCRIPTION

[0022] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognizes that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0023] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0024] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range covering plus or minus up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" can mean within an acceptable error range for the particular value should be assumed.

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[0025] A "unit dose" when used in reference to a pharmaceutical composition can refer to units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect. In some instance the unit dose can contain a diluent; *i.e.*, carrier, or vehicle. In some instances, the unit dose can be a physically discrete.

[0026] The term "saccharide" can refer to a saccharide, oligosaccharide or polysaccharide. For example, saccharide can mean a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexasaccharide, a heptasaccharide, an octasaccharide, a nonasaccharide, or a decasaccharide. An oligosaccharide can refer to two to ten saccharides. A polysaccharide can refer to more than ten saccharides.

[0027] The term "polypeptide" can refer to a single linear chain of amino acids held together by amide bonds. A polypeptide can refer to at least two amino acids. A polypeptide can be an oligopeptide, wherein the term "oligopeptide" can refer to a single chain of from two to twenty amino acids joined by amide bonds. A polypeptide can be a protein, wherein the term "protein" can refer to a single chain of fifty or more amino acids held together by amide bonds.

[0028] The term an "immune response" can refer to the production of cytokines and/or antibodies with a degree of specificity for an antigen after administering the antigen to a subject. In some instances, the term "immune response" can refer to any change in immune cells or change caused by immune cells after administering the antigen to a subject. An immune response can be T cell independent, T cell dependent, or both.

[0029] The term "homology" can mean percent sequence identity between a particular nucleic acid or amino acid sequence and another nucleic acid or amino acid sequence. First, a nucleic acid or amino acid sequence can be compared another sequence using the BLAST 2 Sequences (Bl2seq) program from the stand- alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. BLASTN can be used to compare nucleic acid sequences, while BLASTP can be used to compare amino acid sequences. Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity or homology can be determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (*e.g.*, 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100%.

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[0030] The term "length homology" can mean percent length identity between the length of a particular polypeptide and the length of another polypeptide. "Length homology" can in some instances be calculated by dividing the number of amino acids in a first peptide chain by the number of amino acids in a second peptide chain and multiplying the result by 100%. Chain amino acids can be those forming the backbone of the peptide.

[0031] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0032] The term "immunogenic fragment" can be a fragment that is conjugated to a polypeptide, administered to a subject, and which elicits an immune response. In some instances, the fragment can be a fragment of a capsular polysaccharide. In other instances, the polypeptide can be CRM_{197}.

[0033] Immunogenic, which can but need not be obligated to be preceded any term herein, can mean that which, when administered to a subject, which can be a human or an animal, for example a dog, cat, rat, mouse, sheep, or monkey, produces an immune response in the animal. For example, a dog can be *Canis lupis familiarius*, a cat can be *Felis catus*, a rat can be *Rattus norvegicus*, a mouse can be *Mus musculus*, a sheep can be *Ovis aries*, or a monkey can be *Simia inuus*. The administration can be, for example, subcutaneous, intramuscular, or rectal, for example in the form of a suppository.

[0034] In some embodiments, a unique immunogenic saccharide-polypeptide conjugate can be one that differs from other unique immunogenic saccharide-polypeptide conjugates, for example in a plurality of these, by at least one aspect. For example, the unique immunogenic saccharide-polypeptide conjugate can differ by the saccharide or fragment thereof it contains - in that this can render the unique immunogenic saccharide-polypeptide conjugate unique, or by the polypeptide it contains, or both. In some instances, unique immunogenic saccharide-polypeptide conjugates, to be unique, simply need not be 100% identical.

[0035] The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide.

[0036] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0037] The term "plurality" can mean two or more.

[0038] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

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[0039] Bacteria can incorporate saccharides into their surface structure. For example, *Streptococcus pneumoniae* (*S. pneumoniae*) can contain saccharides in its surface structure. The saccharides can be antigens that can induce an immune response in host, such as an animal or human. For example, B cells can produce antibodies against a saccharide. This response can be a T cell independent immune response. An immune response can be induced with full-length, native form polysaccharides, oligosaccharides, or fragments thereof. An immune response can be induced with full-length, native form capsular polysaccharides, or fragments thereof. Other bacteria that can contain saccharides are *Neisseria meningitidis (N. meningitidis)*, *Salmonella enterica (S. enterica)*, and *Haemophilus influenzae (H. influenzae)*.

[0040] The saccharides on bacteria can be used to classify the bacteria into a subclass or serotype. More specifically, a serotype can refer to the type of a microorganism determined by its constituent antigens. The constituent antigens can be different saccharides, such as different capsular polysaccharides or immunogenic fragments thereof. Some non-limiting examples of *S. pneumoniae* serotypes can include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B).

[0041] By conjugating a saccharide to a polypeptide, the immune response to the saccharide can be enhanced by inducing a T cell dependent response. A saccharide alone may not stimulate T cells because, for example, a saccharide may not be loaded onto the Major Histocompatibility Complex (MHC) of antigen presenting cells. However, peptides can be loaded onto MHC. Therefore, by conjugating a saccharide to a polypeptide, the saccharide-polypeptide conjugate can then be loaded onto the MHC, recognized by the T cell, and stimulate a T cell response. T cells can then stimulate a more vigorous immune response and also promote a more rapid and long-lasting immunologic memory. Provided herein are saccharide-polypeptide conjugates that can be used to induce an immune response, which can be referred to as immunogenic saccharide-polypeptide conjugates. In some instances, the immunogenic saccharide-polypeptide conjugates can provide immune protection against a saccharide or organism containing the saccharides. In other instances, the immunogenic saccharide-polypeptide conjugates can be part of a vaccine.

Compositions of Saccharide-Polypeptide Conjugate

[0042] An immunogenic saccharide-polypeptide conjugate composition can be a saccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition

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can be a capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide or fragment of the capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise a saccharide antigen coupled to one or more polypeptides. An immunogenic saccharide-polypeptide conjugate can comprise one unique saccharide antigen coupled a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise two or more unique saccharide antigens coupled a polypeptide. A saccharide antigen can elicit an immune response.

[0043] A saccharide can be from any bacteria and fungi that can incorporate saccharides into their surface structure. For example, saccharides can be from *S. pneumoniae*. Further examples include saccharides from *N. meningitidis*, *S. enterica*, or *H. influenzae*. A saccharide can be a full-length, native form polysaccharide, oligosaccharide, or fragments thereof. A saccharide can be in any form that induces an immune response. A saccharide can be a capsular polysaccharide or fragment thereof. A saccharide can be from a subclass or serotype. Some non-limiting examples of saccharides from a *S. pneumoniae* serotype include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20. Serogroup 20 can comprise serotype 20A and serotype 20B. *S. pneumoniae* that can produce serotype 20A polysaccharide can be identified by a mutated *whaF* allele as compared to the intact *whaF* allele of *S. pneumoniae* that can produce serotype 20B polysaccharide. **TABLE 1** depicts exemplary repeat unit polysaccharide structures of various *S. pneumonaie* serotypes. **FIGURES 11A, 11B, 11C, 11D**, **11E**, and **11F** depict annotated the capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley et al. Genetic Analysis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

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TABLE 1. Exemplary *S. pneumoniae* Polysaccharide Serotypes

Serotype	Polysaccharide Structure (Repeat Unit)
1	→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→]$_n$ Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→]$_n$ 2 ↓ 1 α-D-Glc*p*A-(1→6)-α-D-Glc*p*
3	→3)-β-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
4	→3)-β-D-Man*p*NAc-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→4)-α-Gal*p*2,3(S)Pyr-(1→]$_n$
5	→4)-β-D-Glc*p*-(1→4)- α -L-Fuc*p*NAc-(1→3)-β-D-Sug*p*-(1→]$_n$ 3 ↑ 1 α-L-Pne*p*NAc-(1-2)-β-D-Glc*p*A L-PneNAc: 2-acetamido-2,6-dideoxy-l-talose (pneumosamine) Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
6A	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitool-(5→P→]$_n$
6B	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Ribitol-(5→P→]$_n$
6C	→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitol-(5→P→]$_n$

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Serotype	Polysaccharide Structure (Repeat Unit)
7F	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 2 4 ↑ ↑ 1 1 β-D-Gal*p* α-D-Gal*p*NAc-(1→2)-α-L-Rha*p*
8	→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→4)-α-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
9N	→4)-α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→]$_n$
9V	→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→]$_n$ ↑ ↑ ↑ 2Ac (17%) 4Ac (6%) 2Ac (3%) 3Ac (25%) 6Ac (55%) 3Ac (4%)
10A	Galβ1 ↓ 6 →4)-GalNAc-β-(1→3)-Gal-α-(1→2)-ribitol-5-PO4$^-$-5Gal*f*β-(1→3)-Galβ-(1→]$_n$ 3 ↑ Gal*f*β1
11A	OAc$_{0.5}$ OAc ↓ ↓ 3 2 →3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→]$_n$ 4 ↑ PO$_4$-1-glycerol

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Serotype	Polysaccharide Structure (Repeat Unit)
	0.5 OAc undeterminate
12F	→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Man*p*NAc-(1→] 3 3 ↑ ↑ 1 1 α-D-Gal*p* α-D-Glc*p*-(1→2)-α-D-Glc*p*
14	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 β-D-Gal*p*
15A	→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-α-Gal*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
15B	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α -D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline Note: On average 70% of the repeating units is O acetylated. The site of the O acetylation is unknown (Jansson *et al.* Carbohydr. Res. (1987) 162:111-116), although Venkateswaran *et al.* Infect. Dis. (1983) 147:1041-1054 suggested that it is linked to a galactose moiety.

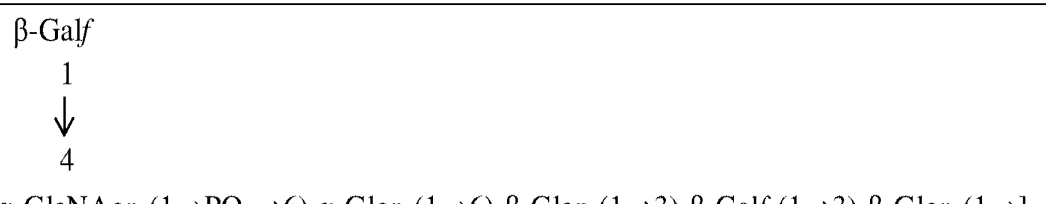
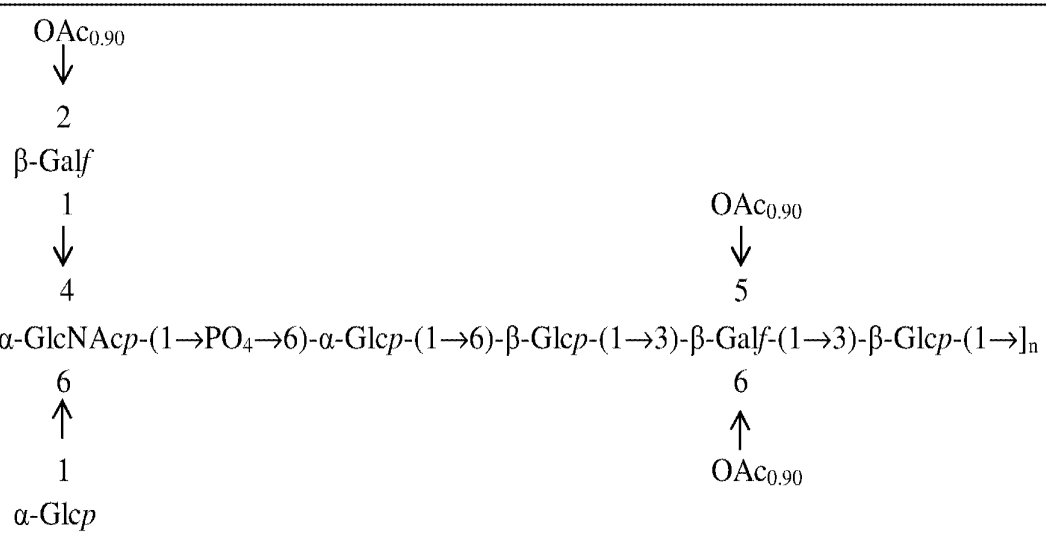
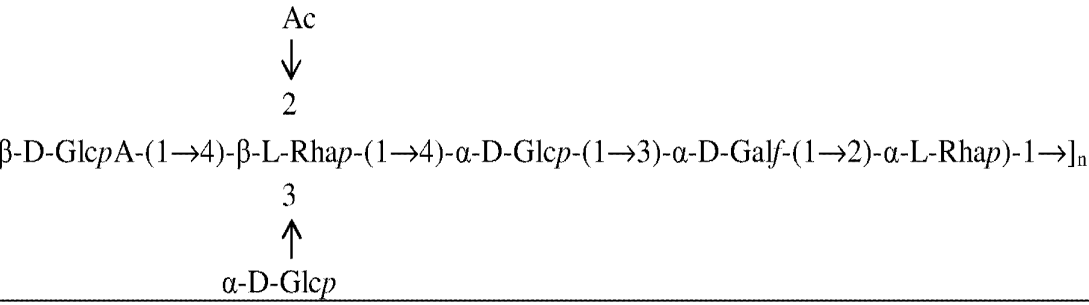
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 4 ↑ 4 β-D-Gal*p* and OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 1 ↑ 4 β-D-Gal*p*

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Serotype	Polysaccharide Structure (Repeat Unit)
18C	α-D-Glc*p* 1 ↓ 2 -[4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rhap-(l→]n 3 ↑ PO4-1-glycerol
19F	→2)-α-L-Rha-(1→5-PO_4^--5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$
19A	→3)-α-L-Rha-(1→5-PO_4^--5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$

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Serotype	Polysaccharide Structure (Repeat Unit)
20A	
20B	
22F	

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Serotype	Polysaccharide Structure (Repeat Unit)
23F	a-L-Rha*p* 1 ↓ 2 →3)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
31	→2)-β-L-Rha*p*-(1→3)-β-D-Gal*f*-(1→3)-β-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→]$_n$
33F	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)– β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*-(1→]$_n$ 2 2 ↑ ↑ 1 $OAc_{0.4}$ α-D-Gal*p*
35F	→6)-Gal*f*-β-(1→3)-Gal-α-(1→2)-Rib-5-PO_4^--3-Gal*f*-β-(1→3)-Gal-β-1→]$_n$ ↓ 2OAc
35B	$OAc_{0.7}$ ↓ 2 →4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*-(1→1)-Rib-(5-PO_4→]$_n$

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[0044] Saccharides can be isolated from bacteria. Saccharides can be isolated from bacteria and then conjugated to a polypeptide to form a saccharide-polypeptide conjugate. Any method for isolating the unconjugated polysaccharide can be used. For example, a polysaccharide serotype can be grown in a soy-based medium. The individual polysaccharides can then be purified by steps including centrifugation, precipitation, and ultra-filtration. *See* U.S. Patent Application Publication No. 2008/028638 and U.S. Pat. No. 5,847,112. Other processes for purifying polysaccharides are described in U.S. Pat. No. 4,686,102; 4,242,501; 4221,906; 5623,057; and 5,847,112. Alternatively or additionally, polysaccharides may be isolated simultaneously from more than one serotype, which can produce a mixture of polysaccharide serotypes with the desired polysaccharide already combined. Furthermore, these isolated polysaccharides can be sized by microfluidization. The polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated sample. A saccharide-polypeptide conjugate can include a saccharide or a fragment thereof which is at least partially embedded in one or more polypeptides. The one or more polypeptides can have one or more crosslinks. The at least partially embedded saccharide or fragment thereof can, but need not be, covalently bound to at least one of the polypeptides when at least partially embedded therein.

[0045] Any of the *S. pneumoniae* strains for isolating and purifying the polysaccharides as described herein can be provided by either the CDC, United States or CCUG, Gothenburg. Alternatively, any of the polysaccharides described herein can be isolated and purified from any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the unique strain from which the polysaccharides are isolated and purified. A vial of a strain of *S. pneumoniae* can be seeded and then can be grown overnight on an agar plate. A single colony can be picked from this plate and streaked onto a new agar plate for overnight growth. This process can be repeated three times to purify the strain before a flask can be seeded and incubated. After allowing the seeded flask sufficient time for growth, the flask contents can be harvested by centrifugation. The centrifugation product can be resuspended in a medium of about 20% glycerol and then can be dispensed into cryogenic vials. The cell bank can be contained in the cryogenic vials, which can be stored at -70°C until use. The cell bank of the strain can then be used for isolation and purification of the polysaccharides of that strain using a fermentation process.

[0046] Polysaccharides of a *S. pneumoniae* strain can be isolated and purified from a cell bank of a strain. The strain can be provided by either the CDC, United States or CCUG, Gothenburg, and then can be used to produce a cell bank as described above. Alternatively,

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strain can be provided by any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the serotype of the strain. A vial of a cell bank can be produced as described above is used to inoculate a flask, which is allowed to grow overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 μm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating). Any polysaccharides described herein can be isolated and purified using this method.

[0047] The saccharides or polysaccharides of the invention are depolymerized (sized) to a final range of 100-500 kDa.

[0048] Saccharides that can be used in the saccharide-polypeptide conjugate can also be obtained from commercial sources. For example, individual polysaccharide serotypes can be obtained in a powder form. These polysaccharides can be dissolved in water and incubated with a salt to dissociate residual impurities, which can then be removed by a filtration step. The purified polysaccharides serotypes can then be conjugated to a polypeptide.

[0049] A polypeptide of an immunogenic saccharide-polypeptide conjugate can be an oligopeptide. A polypeptide of can be a carrier protein. A polypeptide can be any polypeptide that allows for conjugation or coupling of saccharide and results in the display of the conjugate or coupling in a way that induces a protective immune response against the saccharide. A polypeptide can be any polypeptide that allows for conjugation or coupling of a capsular polysaccharide or fragment of the capsular polysaccharide and can result in the display of the conjugate or coupling in a way that can induce a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide. A polypeptide can be CRM_{197}, tetanus toxoid, a diphtheria toxoid, a cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, a keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, or any fragment thereof. The polypeptide can be a polypeptide in which the

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toxin activity of the polypeptide can be at least partially mitigated. Examples of suitable polypeptides for use as in a saccharide-polypeptide conjugate are described in U.S. patent 8,808,707 and can include: polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, *e.g.*, serines, threonines, or tyrosines, to confer desirable solubility properties on the resultant loaded carrier and conjugate. A polypeptide can be a CRM_{197} with 100% sequence homology with GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). A polypeptide can be a CRM_{197} with at least 80%, 90%, 95%, or 98% sequence homology with SEQ ID NO: 1. A polypeptide can be a modified CRM_{197} as disclosed in US Patent Application No. 14/329,758. A polypeptide can be CRM_{197} with non-natural amino acid substitutions as disclosed in US Patent Application No. 14/328,532.

[0050] Moreover, suitable polypeptide carriers can include bacterial toxins, toxoids, or inactivated toxin. A toxoid can be a bacterial toxin whose toxicity has been weakened or suppressed while other properties, typically immunogenicity, are maintained. As a class, bacterial toxins and derivatives thereof can to be highly immunogenic. Polypeptides derived from bacterial toxins can be effective at eliciting an immune response to saccharides of the conjugate. Steps can be taken (*e.g.*, by chemical and/or genetic means) to render the toxins non-toxic and safe for administration to mammals. Examples of such bacterial toxin-derived polypeptides which may currently commonly used in vaccine compositions, and can be used in the immunogenic saccharide-polypeptide conjugate described herein, include the diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from

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N. meningitidis, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, or any fragment thereof. Polypeptide fragments can be used to elicit a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide of the immunogenic saccharide-polypeptide conjugate. These fragments can encompass T-helper epitopes.

[0051] CRM_{197} is a non-toxic mutant of diphtheria toxin with a single amino acid substitution of glutamic acid for glycine at position 52, with the amino acid sequence of GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). CRM_{197} can be from 58-59 kDa in size. CRM_{197} can be 58.4 kDa in size. CRM_{197} can be produced, isolated, and purified from a cell bank. A vial from a cell bank of CRM_{197} can be used to seed a new flask and allowed to ferment. The fermentation culture can be centrifuged and the contents can be harvested, clarified, and filtered using a 0.2 μm filter. The filtrate can then be concentrated and diafiltered. The CRM_{197} can be further purified by filtration using microtubes or membranes of the appropriate pore size. Then anion exchange chromatography can be used in the isolation and purification of the CRM_{197}, which can be followed by ammonium sulphate precipitation, and another filtration using a 0.45 μm filter. CRM_{197} can be further isolated and purified from this product by hydrophobic interaction chromatography, after which CRM_{197} can be concentrated and diafiltered into the desired buffer. A final step can be filtration with 0.2 μm filter. The isolated and purified CRM_{197} can be stored at ≤ -70°C until use in conjugation with a saccharide or polysaccharide. Quality control tests can performed on the isolated and purified CRM_{197}.

[0052] A polypeptide can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 2 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be

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conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 17F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A

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polypeptide can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.*

[0053] In some embodiments, CRM_{197} can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae.* CRM_{197} can be conjugated to an immunogenic serotype 2 capsular

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polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 17F capsular polysaccharide,

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fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*.

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[0054] In further embodiments, any of the saccharides as described herein can be conjugated to any polypeptides described herein. Any of the immunogenic saccharide-polypeptide conjugates described herein can be isolated and purified.

Methods of Conjugating a Saccharide and Polypeptide to Produce a Saccharide-Polypeptide Conjugate

[0055] The saccharides can be conjugated to the polypeptide by, *e.g.*, chemical or biological synthesis. A saccharide can be a polysaccharide, a capsular polysaccharide, or an immunogenic fragment thereof. A capsular polysaccharide or fragment of the capsular polysaccharide can be conjugated to the polypeptide by chemical or biological synthesis.

[0056] For example, the capsular polysaccharide or immunogenic fragment thereof can be chemically activated and then reacted with the polypeptide. For example, capsular polysaccharides or fragments of the capsular polysaccharides can be reacted with either ammonia or diaminoethane to generate a free terminal amino group. The amino group can then be reacted with an excess of disuccinimidyl ester of adipic acid, which can introduce an active succininmidyl ester group. This can then be reacted to a free amino group on the polypeptide to form covalent amide bonds.

[0057] A polysaccharide and polypeptide can be conjugated using 1-cyano-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry. CDAP can be used to activate a broad range of acidic, neutral, and other classes of polysaccharides, which can then be derivatized with diamines, dihydrazides or other functional groups in preparation for coupling to proteins. In CDAP, the cynanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate can be used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which can avoid hydrolysis of the alkaline sensitive polysaccharides. This synthesis can allow for direct coupling to a carrier. The polysaccharide can be solubilized in water or a saline solution. CDAP can be dissolved in acetonitrile and can be added immediately to the polysaccharide solution. The CDAP can reacts with the hydroxyl groups of the polysaccharide to form a cyanate ester. This cyanate ester can be highly reactive, which can result in an activated polysaccharide. This can be done at a pH of 9–10. After this activation step, the polypeptide can be added. Amino groups of lysine of a polypeptide can react with the activated polysaccharide to form an isourea covalent link. This conjugation step can occur under mildly alkaline conditions, such as a pH of 7–9. In some cases, there can be a strong pH dependence on the epsilon amines of lysine to form a stable O-alkyl-isourea linkage. After the coupling

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reaction, a large excess of an amino-containing reagent such as glycine can then be added to quench the residual activated functional groups. The CDAP activation can take only a few minutes and conjugation can be completed in a few hours. The conjugate can then be passed through a gel permeation column to remove unreacted polypeptide and residual reagents.

[0058] In some embodiments, CDAP can be made up at 100 mg/ml in acetonitrile and stored at -20°C for up to 1 month. CDAP can be slowly pipetted into a vortexed solution of polysaccharide in water (rapid addition of the organic co-solvent precipitates the polysaccharide) and 20 s later a volume of aqueous 0.2M triethylamine (TEA) equal to volume of CDAP used can be added. At 2.5 min, a large molar excess of a 0.5 M hexanediamine solution in either 0.1 M sodium borate at pH 9.3 or 0.75 M HEPES at pH 7.5, can be added. The reaction mixture can be allowed to stand overnight at 4°C, can be desalted on either a P-6 DG or a P-6 cartridge, can be equilibrated with saline and then can be further dialyzed into saline. The extent of derivatization with hexanediamine can be determined using a TNBS assay for primary amines; absorbance can be measured at 366nm, using an extinction coefficient of 11000 M^{-1}. Polysaccharide concentrations can be determined as described by Monsigny et al., using the corresponding polysaccharide as the standard.

[0059] In some embodiments, polysaccharide can be solubilized in saline at 10 mg/ml. At t = 0, CDAP (100 mg/ml in acetonitrile) can be added (1 mg CDAP/ mg polysaccharide) while vortexing. At 30 s, 0.2 M TEA can be added (10 µl/mg polysaccharide) to raise the pH. At 2.5 min the pH can be readjusted to the coupling pH and the amine or hydrazide reagent or the polypeptide can be added, in the appropriate buffer. After an overnight reaction, the solution can be dialyzed and/or desalted on a P6DG column. Polysaccharide polypeptide conjugates can be fractionated on an S400HR column, which can be equilibrated with either saline or PBS. HPLC can be performed on HPLC size exclusion columns using a Waters 626 pump, monitored at 280 nm and with a refractive index detector. Samples can be filtered before chromatography using a Millex Ultrafree MC device.

[0060] In some embodiments, for low pH coupling, amines on polypeptide can be functionalized with hydrazides using the two-step method as follows. Five mg of polypeptide at 12.5 mg/ml in 0.19 M HEPES, 2.5 mM EDTA, pH 7.3 can be iodoacetylated by the addition of a 15-fold molar excess of SIA over moles of polypeptide. After 2 h reaction in the dark, the solution can be desalted on a 1 x 15 cm P6DG column, can be equilibrated with 2 mM MES, 0.2 M NaCl, pH 5.9 and the void volume fractions, which can be concentrated with a Centricon 50 device. DTPH (30 µl) at 0.1 M in water, can be reduced to 3-mercapto-propionyl hydrazide by addition of 25 µl 0.1 M TCEP in water, and can then be added to the

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iodoacetylated polypeptide with a pH that can be adjusted to about 5. After a 2 h reaction, reagent can be removed by desalting again and concentrating with a Centricon 50 device to 10 mg/ml. TNBS can be used to determined number of hydrazide per moles of polypeptide. To couple the hydrazide-derivatized polypeptide, 3 mg of polysaccharide at 10 mg/ml in saline can be activated by the addition of 15 µl CDAP at 100 mg/ml in acetonitrile, followed 30 s later by 30 µl of 0.2 M TEA. At 2.5 min, the pH can be reduced to 5.5 by the addition of 100 µl 1 M MES, pH 5.5 and the activated polysaccharide can be added to 3.8 mg of the polypeptide-hydrazide. After an overnight reaction at 4°C, the reaction can be quenched by the addition of 50 µl 1 M ethanolamine. The solution can be applied to an S400HR column, which can be equilibrated with PBS, and the void volume fractions can be pooled. Sterile filtration can be done by passage through a Millex GB 0.2 µm filter.

[0061] In some embodiments, CDAP can be used to conjugate a polysaccharide and CRM_{197}. The polysaccharide (10 mg) can be dissolved in 1.0 mL of 0.1M sodium borate buffer pH 9.0 and can be activated by addition of 50 µl of a solution of CDAP dissolved in acetone:water (6:4). Immediately, the pH can be adjusted and maintained at 9.0 by continuous dropwise addition of a 0.1 M NaOH solution. The mixture can be stirred for 5 min and then a volume of 1.4 mL of 0.05 M HCl can be added, stirred for a while and then left to rest during 1 ½ min more. The mixture can be rapidly passed through a PD-10 column equilibrated in 0.1 M sodium bicarbonate buffer pH 8.3. An aliquot of this can be used to determine cyanate ester formation and the rest of the preparation can be immediately used for coupling purposes. Polypeptide (15 mg) can be dissolved in 1.0 mL of 0.1 M $NaHCO_3$ buffer pH 8.3 and added to the activated polysaccharide solution. The mixture can be gently stirred during 8 h at room temperature. A volume of 1.0 mL 0.1 M Tris-HCl buffer pH 8.6 can be added to block any remaining activation sites on the unreacted polysaccharide and incubated during 1 h more. The final solution can be lyophilized and stored until used for the purification procedure. The progress of the conjugation can be analyzed by high-performance size exclusion chromatography (HPSEC) using a Zorbax GF-250 column and monitored at 280 nm and 210 nm. For example, aliquots of the reaction mixture can be taken at 10 min and at every hour up to 4 h. The column can be equilibrated in 0.025 M phosphate buffer pH 7.0 supplemented with 0.13 M NaCl and be calibrated with the following proteins: thyroglobulin(669 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), BSA (67 kDa), ovalbumin (43 kDa), and ribonuclease (13.7 kDa). The void volume can be determined with Blue dextran. Samples (10 µl aliquots) can be injected into the column and a flow rate of 0.5 mL/min can be used. Prior and after the analysis the column can be washed with the equilibration buffer.

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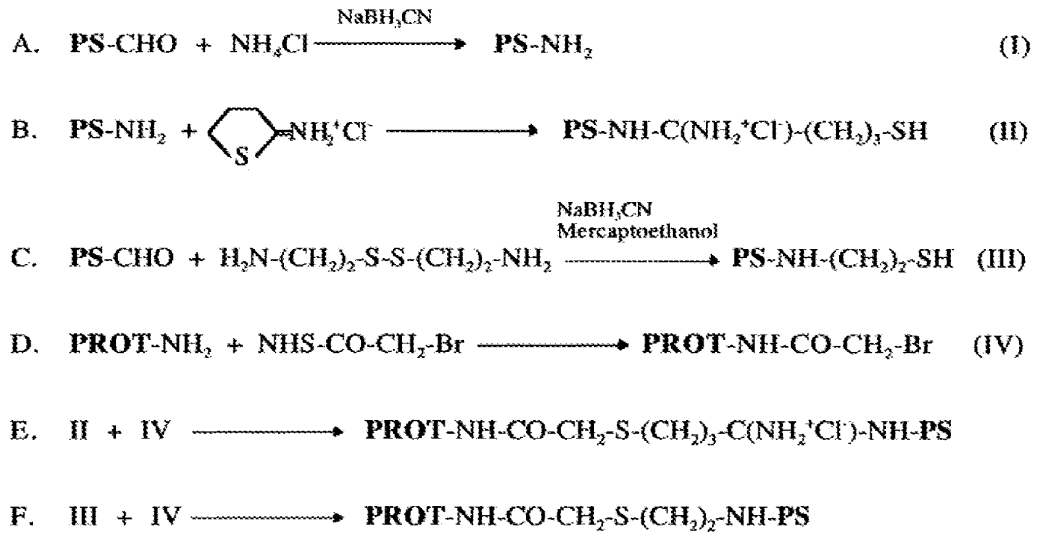
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[0062] In some embodiments, the polysaccharide can be activated via CDAP chemistry in a CDAP solution. The polysaccharide can be mixed with CDAP solution, which can then be incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} can be added to the activated polysaccharides. The reaction can then be quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture can be filtered, diafiltered, and concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates can then be purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates can be pooled, concentrated, and then filtered through a 0.2 μm filter. The product can be stored at 2-8°C.

[0063] As another example, the capsular polysaccharide or immunogenic fragment thereof can activated by periodate oxidation. Sodium periodate oxidizes diols (two adjacent carbons with hydroxyl groups) can be converted into aldehydes (C=O) and in the process breaks C-C bonds. Thus, depending upon the structure, periodate activation can fragment a capsular polysaccharide or immunogenic fragment thereof and open the ring structures of sugars. When the diol is within a ring, the ring sugar can be opened possibly altering the polysaccharide conformation. When the diol is in a glycerol or ribitol side chain, the side chain can disappear. The reactive aldehyde groups can then condense with free amino groups on the polypeptide in the presence of sodium cyanoborohydride to form a stable secondary amine. Condensation of the aldehyde with the epsilon amino group on lysine can be a slow process and can take a few days. The remaining aldehyde groups can then be quenched using sodium borohydride, which can convert them back to hydroxyls.

[0064] Another example of coupling a polypeptide to a polysaccharide is based on thioether bonding of polysaccharides activated with 2-iminothiolane (2-IT), or alternatively, with cystamine, to bromoacetylated polypeptides such as tetanus toxoid.

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Schematic I

[0065] The above Schematic I represents end-point coupling of polysaccharides (PS) to proteins (PROT) through thioether bond. (A) Amination of polysaccharide through reducing terminal using ammonium chloride in the presence of cyanoborohydride ion. (B) Thiolation of aminated polysaccharide (I) with 2-iminothiolane (2-IT). (C) Alternative thiolation of polysaccharide through reductive amination with cystamine with concomitant cleavage of disulfide bond. (D) Bromoacetylation of amino groups of protein using N-hydroxysuccimidyl bromoacetate. (E) Coupling of polysaccharide derivatized with 2-IT (II) to bromoacetylated protein (IV). (F) Alternative coupling of polysaccharide derivatized with cystamine (III) to bromocacetylated protein (IV).

[0066] As another example, the immunogenic saccharide-polypeptide conjugate can be produced using bacteria, such as *Escherichia coli* (*E. coli*). The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially embedded in the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide chemically cross-linked to the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially chemically cross-linked to the polypeptide. A capsular polysaccharide or fragment of the capsular polysaccharide of a serotype from *S. pneumoniae* can be conjugated to a polypeptide by chemical or biological synthesis. One saccharide can be conjugated to one polypeptide. Two or more saccharides can be conjugated to one polypeptide. The saccharides can be the same serotype. The saccharides can be different serotypes. One capsular polysaccharide or immunogenic fragment thereof can be conjugated to one polypeptide. Two or more capsular polysaccharides or fragments thereof can be conjugated to one polypeptide. The capsular

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polysaccharides or fragments thereof can be the same serotype. The capsular polysaccharides or fragments thereof can be different serotypes.

[0067] After conjugation, the polysaccharide polypeptide conjugate can be purified. For example, different chromatographic strategies can be used to purify a polysaccharide-polypeptide conjugate: SEC (using a column packed with Sepharose CL-6B), a combination of ion-exchange chromatography (DEAE-Sepharose) can be followed by HPSEC (Sepharose CL-6B and hydrophobic interaction chromatography (HIC) (column packed with Sephadex LH-20). For HPSEC purification on a Sepharose CL-6B column, an amount of 4.0 mg of the lyophilized mixture containing the polysaccharide polypeptide conjugate, free polysaccharide and free polypeptide, can be dissolved in 0.1 M formic acid buffer (ammonium salt) pH 6.0 and can be applied to the column (60 cm x 4.0 cm) equilibrated with the same buffer, at a flow of 0.5 mL/min. Isocratic elution can be performed with the buffer, and the process can be continuously monitored using a differential refractometric detector. Fractions can be collected, can be lyophilized, and can be used for further analysis. For the second strategy, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 500 µL of 0.1 M phosphate-buffered saline (PBS) pH 7.0 and can be applied to the DEAE-Sepharose column, previously equilibrated in the same buffer. The pass-through of the column can be collected and after washing with the buffer, elution can be performed using a gradient (0.25-1.0 M) of NaCl. The presence of the polysaccharide can be analyzed in each fraction by the Phenol sulfuric technique. The eluted fractions can be pooled, can be lyophilized, can be resuspended in 0.1 M ammonium formate buffer pH 6.0, and can be applied to a Sepharose CL-6B column. Fractions of 5 mL can be collected, can be dialyzed, can be lyophilized, and can be analyzed for protein and carbohydrate contents. In a third chromatographic approach, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 1.0 mL of distilled water and applied to a column packed with Sephadex LH-20 previously equilibrated with distilled water, at a flow rate of 0.5 mL/min. Elution can be performed isocratically with distilled water, and the process can be monitored as described above. Fractions can be collected, can be lyophilized, and can be used for further analysis.

[0068] Furthermore, 'click' chemistry can be used to conjugate a saccharide with a polypeptide as disclosed in US Patent No. 8,753,645 and in Hu et al., Towards the next generation of biomedicines by site selective conjugation, *Chem. Soc. Rev.*, 2016, 45, 1691. 'Click' chemistry can be used to conjugate modified CRM_{197} or CRM_{197} with non-natural amino acids to polysaccharides.

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Pharmaceutical compositions

[0069] The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition. The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition in unit dose form. The saccharide-polypeptide conjugates described herein can be in the form of a vaccine. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can be in unit dose form and can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate wherein the capsular polysaccharide or immunogenic fragment thereof of the saccharide-polypeptide conjugate can be one serotype. In another embodiment, a pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the capsular polysaccharides or immunogenic fragments thereof can be from different serotypes, creating a mixture of different immunogenic saccharide-polypeptide conjugates. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the polypeptide is the same polypeptide in all the immunogenic saccharide-polypeptide conjugates. In other embodiments, a pharmaceutical composition can comprise immunogenic saccharide-polypeptide conjugates wherein the polypeptide(s) in the saccharide-polypeptide conjugates are a mixture of different polypeptide(s).

[0070] For example, a pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide,

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fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of at least eight immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31,34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes

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selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, , 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide

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conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide

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conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 34F, 31, 33B,34, 35F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 20B, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, 38 or

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Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at

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least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes

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comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at

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least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates

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comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising

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serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20

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(comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F,

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24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. The immunogenic saccharide-polypeptide conjugate(s) can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include excipients that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include adjuvants that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The pharmaceutical composition can be administered to one of a plurality of humans, which elicits at least one of the following side effects in less than about 5%, 10%, 20% or 30% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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[0071] In other embodiments, a pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide is from *N. meningitidis*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *S. enterica*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *H. influenzae*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *Neisseria meningitidis*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *S. enterica*. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *H. influenzae*.

[0072] The polypeptide of any of the plurality of saccharide-polypeptide conjugates can be the same polypeptide. For example, the polypeptide of any of the plurality of saccharide-polypeptide conjugates can be CRM_{197}. The plurality of saccharide-polypeptide conjugates can comprise a mixture of different polypeptides. For example, the plurality of saccharide-polypeptide conjugates can comprise a mixture of CRM_{197}, tetanus toxoid, a diphtheria toxoid, or any fragment thereof.

[0073] Any pharmaceutical composition described herein can be in unit dose form. Additionally, the pharmaceutical composition can further comprise an adjuvant. In some embodiments, the adjuvant can be an aluminum-cation comprises adjuvant. In other embodiments, the adjuvant can be aluminum phosphate, aluminum sulfate, or aluminum hydroxide. More examples of suitable adjuvants for use in the pharmaceutical composition include: aluminum-based salts and variants thereof; emulsions (either water-in-oil or oil-in-water) and variants thereof, *e.g.*, Freund's Incomplete Adjuvant, MF59® (Glaxosmithkline plc); PRR ligands and variants thereof, *e.g.*, pathogen associated molecular patterns (PAMPs); TLR3 and RLR ligands or variants thereof, *e.g.*, Synthetic analogs of dsRNA, poly(I:C), etc.; TLR4 ligands or variants thereof, *e.g.*, bacterial flagellin, Glucopyranosyl Lipid Adjuvant (GLA), monophosphoryl lipid A, etc.; TLR5 ligands or variants thereof, *e.g.*, imiquimod, gardiquimod, R848, etc.; TLR9 ligands or variants thereof, *e.g.*, Oxydeoxynucleotides containing CpG motifs (CpG ODNs such as ODN1826 and ODN2006); NOD2 ligands or variants thereof, *e.g.*, fragments of bacterial cell walls (such as muramyl dipeptide [MDP]); saponins including synthetic derivatives or variants thereof, e.g., QS21, TQL1055, etc.; or any combination of above. An adjuvant can be 3M-052.

[0074] A pharmaceutical composition can further comprise an excipient. An excipient can be Acacia, Acesulfame Potassium, Acetic Acid, Glacial Acetone, Acetyltributyl Citrate,

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Acetyltriethyl Citrate, Agar, Albumin, Alcohol, Alginic Acid, Aliphatic Polyesters, Alitame, Almond Oil, Alpha Tocopherol, Aluminum Hydroxide Adjuvant, Aluminum Oxide, Aluminum Phosphate Adjuvant, Aluminum Stearate, Ammonia Solution, Ammonium Alginate, Ascorbic Acid, Ascorbyl Palmitate, Aspartame, Attapulgite, Bentonite, Benzalkonium Chloride, Benzethonium Chloride, Benzoic Acid, Benzyl Alcohol, Benzyl Benzoate, Boric Acid, Bronopol, Butylated Hydroxyanisole, Butylated Hydroxytoluene, Butylparaben, Calcium Alginate, Calcium Carbonate, Dibasic Anhydrous Calcium Phosphate, Dibasic Dihydrate Calcium Phosphate, Tribasic Calcium Phosphate, Calcium Stearate, Calcium Sulfate, Canola Oil, Carbomer, Carbon Dioxide, Carboxymethylcellulose Calcium, Carboxymethylcellulose Sodium, Carrageenan, Castor Oil, Hydrogenated Castor Oil, Microcrystalline Cellulose, Powdered Cellulose, Silicified Microcrystalline Cellulose, Cellulose Acetate, Cellulose Acetate Phthalate, Ceratonia, Cetostearyl Alcohol, Cetrimide, Cetyl Alcohol, Cetylpyridinium Chloride, Chitosan, Chlorhexidine, Chlorobutanol, Chlorocresol, Chlorodifluoroethane (HCFC), Chlorofluorocarbons (CFC), Chloroxylenol, Cholesterol, Citric Acid Monohydrate, Colloidal Silicon Dioxide, Coloring Agents, Copovidone, Corn Oil, Cottonseed Oil, Cresol, Croscarmellose Sodium, Crospovidone, Cyclodextrins, Cyclomethicone, Denatonium Benzoate, Dextrates, Dextrin, Dextrose, Dibutyl Phthalate, Dibutyl Sebacate, Diethanolamine, Diethyl Phthalate, Difluoroethane (HFC), Dimethicone, Dimethyl Ether, Dimethyl Phthalate, Dimethyl Sulfoxide, Dimethylacetamide, Disodium Edetate, Docusate Sodium, Edetic Acid, Erythorbic Acid, Erythritol, Ethyl Acetate, Ethyl Lactate, Ethyl Maltol, Ethyl Oleate, Ethyl Vanillin, Ethylcellulose, Ethylene Glycol Palmitostearate, Ethylene Vinyl Acetate, Ethylparaben, Fructose, Fumaric Acid, Gelatin, Glucose, Liquid, Glycerin, Glyceryl Behenate, Glyceryl Monooleate, Glyceryl Monostearate, Glyceryl Palmitostearate, Glycofurol, Guar Gum, Hectorite, Heptafluoropropane (HFC), Hexetidine, Hydrocarbons (HC), Hydrochloric Acid, Hydroxyethyl Cellulose, Hydroxyethylmethyl Cellulose, Hydroxypropyl Cellulose, Low-substituted Hydroxypropyl Cellulose, Hydroxypropyl Starch, Hypromellose, Hypromellose Acetate Succinate, Hypromellose Phthalate, Imidurea, Inulin, Iron Oxides, Isomalt, Isopropyl Alcohol, Isopropyl Myristate, Isopropyl P,almitate, Kaolin, Lactic Acid, Lactitol, Anhydrous Lactose, Monohydrate Lactose, Spray-Dried Lactose, Lanolin, Lanolin Alcohols, Hydrous Lanolin, Lauric Acid, Lecithin, Leucine, Linoleic Acid, Macrogol, Hydroxystearate Magnesium Aluminum Silicate, Magnesium Carbonate, Magnesium Oxide, Magnesium Silicate, Magnesium Stearate, Magnesium Trisilicate, Malic Acid, Maltitol, Maltitol Solution, Maltodextrin, Maltol, Maltose, Mannitol, Medium-chain Triglycerides, Meglumine, Menthol,

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Methylcellulose, Methylparaben, Mineral Oil, Light Mineral Oil, Mineral Oil and Lanolin Alcohols, Monoethanolamine, Monosodium Glutamate, Monothioglycerol, Myristic Acid, Neohesperidin Dihydrochalcone, Nitrogen, Nitrous Oxide, Octyldodecanol, Oleic Acid, Oleyl Alcohol, Olive Oil, Palmitic Acid, Paraffin, Peanut Oil, Pectin, Petrolatum, Petrolatum and Lanolin Alcohols, Phenol, Phenoxyethanol, Phenylethyl Alcohol, Phenylmercuric Acetate, Phenylmercuric Borate, Phenylmercuric Nitrate, Phosphoric Acid, Polacrilin Potassium, Poloxamer, Polycarbophil, Polydextrose, Polyethylene Glycol, Polyethylene Oxide, Polymethacrylates, Poly(methyl vinyl ether/maleic anhydride), Polyoxyethylene Alkyl Ethers, Polyoxyethylene Castor Oil Derivatives, Polyoxyethylene Sorbitan Fatty Acid Esters, Polyoxyethylene Stearates, Polyvinyl Acetate Phthalate, Polyvinyl Alcohol, Potassium Alginate, Potassium Benzoate, Potassium Bicarbonate, Potassium Chloride, Potassium Citrate, Potassium Hydroxide, Potassium Metabisulfite, Potassium Sorbate, Povidone, Propionic Acid, Propyl Gallate, Propylene Carbonate, Propylene Glycol, Propylene Glycol Alginate, Propylparaben, 2-Pyrrolidone, Raffinose, Saccharin, Saccharin Sodium, Saponite, Sesame Oil, Shellac, Simethicone, Sodium Acetate, Sodium Alginate, Sodium Ascorbate, Sodium Benzoate, Sodium Bicarbonate, Sodium Borate, Sodium Chloride, Sodium Citrate Dihydrate, Sodium Cyclamate, Sodium Hyaluronate, Sodium Hydroxide, Sodium Lactate, Sodium Lauryl Sulfate, Sodium Metabisulfite, Dibasic Sodium Phosphate, Monobasic Sodium Phosphate, Sodium Propionate, Sodium Starch Glycolate, Sodium Stearyl Fumarate, Sodium Sulfite, Sorbic Acid, Sorbitan Esters (Sorbitan Fatty Acid Esters), Sorbitol, Soybean Oil, Starch, Pregelatinized Starch, Sterilizable Maize Starch, Stearic Acid, Stearyl Alcohol, Sucralose, Sucrose, Compressible Sugar, Confectioner's Sugar, Sugar Spheres, Sulfobutylether b-Cyclodextrin, Sulfuric Acid, Sunflower Oil, Hard Fat Suppository Bases, Talc, Tartaric Acid, Tetrafluoroethane (HFC), Thaumatin, Thimerosal, Thymol, Titanium Dioxide, Tragacanth, Trehalose, Triacetin, Tributyl Citrate, Triethanolamine, Triethyl Citrate, Vanillin, Hydrogenated Vegetable Oil, Water, Anionic Emulsifying Wax, Carnauba Wax, Cetyl Esters Wax, Microcrystalline Wax, Nonionic Emulsifying Wax, White Wax, Yellow Wax, Xanthan Gum, Xylitol, Zein, Zinc Acetate, or Zinc Stearate.

[0075] A pharmaceutical composition can further comprise a surfactant or emulsifier, or a combination thereof. A surfactant can be polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. In some embodiments, polysorbate can be polysorbate 80. In some embodiments, polymer glycol can be polyethylene glycol. A surfactant can be TWEEN. A pharmaceutical composition can comprise a specific polyethylene glycol based upon its molecular weight. A pharmaceutical composition can comprise a preservative. A

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pharmaceutical composition can comprise a salt. The salt is an inorganic salt. A pharmaceutical composition can further comprise an anti-fungal compound or a salt thereof. An antifungal compound can be thimerosal, phenol, benzethonium chloride, or 2-phenoxyethanol. A pharmaceutical composition can further comprise a chelating agent. A chelating agent can be ethylenediaminetetraacetic acid (EDTA). A pharmaceutical composition can further comprise a buffering agent. The buffering agent is succinate buffer agent, tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, phosphate buffering agent, sodium phosphate buffering agent, or a potassium phosphate buffering agent. A pharmaceutical composition can further comprise sterile water. When the pharmaceutical composition can be administered intramuscularly to a human, the pharmaceutical composition can elicit an immune response that lasts from about 1 week to about 30 years. A pharmaceutical composition retains at least about 60% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 70% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 90% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0076] The polysaccharide-polypeptide conjugates as described herein can be adjuvanted. Suitable adjuvants can include an aluminum hydroxide gel (alum) or aluminum phosphate, but can also be a salt of calcium, magnesium, iron or zinc, or can be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized polysaccharides, or polyphophazenes. An adjuvant can be 3M-052. When adjuvanted with aluminum salts, the ratio of aluminum salt to polysaccharide can be less than 10:1 (w/w), less than 8:1(w/w), or more than 2:1(w/w) in a vaccine dose or formulation.

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[0077] The amount of polypeptide in a vaccine dose can be selected as an amount which can induces an immunoprotective response without significant adverse effects. A dose or formulation can comprise 0.1-100 μg of polysaccharide and/or for a polysaccharide polypeptide conjugate 0.1-50 μg of polysaccharide. A polysaccharide-polypeptide conjugate can comprise 1-10 μg of polysaccharide, 1-5 μg of polysaccharide, or 2-5 μg of polysaccharide. The content of polypeptide in the vaccine can be in the range of 1-100 μg of polypeptide, 5-50 μg of polypeptide, or 5-25 μg of polypeptide.

[0078] The vaccine dosage can be in the range of 1-5 μg of polysaccharide per polysaccharide polypeptide conjugate delivered intramuscularly as a single 0.5 mL injection. The vaccine dosage can comprise aluminum phosphate (1 mg/mL) and 0.01% polysorbate 80.

Methods of Use

[0079] The saccharide-polypeptide conjugates as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be used in a vaccine. The vaccine can be administered to a subject. The vaccine can be used as a prophylatic for disease caused by *S. pneumoniae*, such as meningitis, pneumonia, and severe invasive diseases associated with pneumococcal infection. The subject can be a human. The subject or human can be a subject or human in need thereof.

[0080] The saccharide-polypeptide conjugate can be administered by intramuscular injection. The saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The saccharide-polypeptide conjugate can be administered via a suppository. The saccharide-polypeptide conjugate can be administered intranasally. The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the saccharide-polypeptide conjugate.

[0081] The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered by intramuscular injection. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary,

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respiratory or genitourinary tracts. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered intranasally. The pharmaceutical composition can be administered intramuscularly and can elicit an immune response that lasts from 1 week to 30 years. The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the pharmaceutical composition comprising the saccharide-polypeptide conjugate.

[0082] For example, a pharmaceutical composition administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, or of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least

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fourteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eighteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). For example, a pharmaceutical composition administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

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23, or of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fourteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least eighteen serotypes selected from

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the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising 20A and 20B).A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition administered to a subject can

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comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F,

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34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F,

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34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B) or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B).A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise

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administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition administered to a subject can comprise can comprise a

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plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.

[0083] Each dosage can be formulated in 0.5 mL. The formulation can comprise normal saline. The formulation can have a pH of 6.5. The formulation can be a pH from 6.3 to 6.7 or from 6.2 to 6.8. The formulation can have a pH of 5.8. The formulation can be a pH from 5.5 to 6.1 or from 5.6 to 6.0. The formulation can comprise 0.001% to 0.01% polysorbate 80.

[0084] Two different pharmaceutical compositions can be administered to a subject. The first pharmaceutical composition can be administered to the subject at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years before or at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years after an administration to the subject a second pharmaceutical composition. The first pharmaceutical composition can be administered to a subject who is at least 4 weeks old, 6 weeks old, 12 weeks old, 24 weeks old, 6 months old, 8 months old, 10 months old, 12 months old, 14 months old, 15 months old, 16 months old, 17 months old, 18 months old, 19 months old, 20 months old, 21 months old, 22 months old, 23 months old, 2 years old or older. The first pharmaceutical composition can be administered to a subject with an age from 4 weeks to 2 years old. The first pharmaceutical composition can be administered to a subject with an age from 18 years old to 100 years old. The first pharmaceutical composition can be administered to a subject with an age of 100 years old or older. In some embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide

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conjugate comprising a polypeptide that has at less than 70% length homology, 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0085] In some embodiments, the first pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). In some embodiments, the first pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); or a combination thereof. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B). The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F,34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B).The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A,

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11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the

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serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. In other embodiments, the first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. When administering any of the first pharmaceutical compositions described above, the first pharmaceutical composition can be distinct from the second pharmaceutical composition administered to a subject.

[0086] In some embodiments, the second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and

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Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates

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comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B,

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33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct.

[0087] In other embodiments, the first pharmaceutical composition and the second pharmaceutical composition can be administered at least about 4 weeks apart, at least 6 about weeks apart, at least 2 about months apart, at a least about 3 months apart, at least about 4 months apart, at least about 5 months apart, at least 6 months apart, at least about 7 months apart, at least about 8 months apart, at least about 9 months apart, at least about 10 months apart, at least 11 about months apart, at least about 1 year apart, at least about 1.5 years apart, at least about 2 years apart, at least about 5 years apart, at least about 10 years apart, at least about 15 year, at least about 20 years apart, or at least about 30 years apart.

[0088] In some embodiments, a serotype of the first pharmaceutical composition can be from a different bacteria than the serotype of the second pharmaceutical composition. For example, the serotype of the first composition can be from *Streptococcus pneumoniae*, and the serotype of the second composition can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. The serotype of the first pharmaceutical composition can be from

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Neisseria meningitidis, *Salmonella enterica*, or *Haemophilus influenzae*, and the serotype of the second pharmaceutical composition can be from *Streptococcus pneumoniae*.

[0089] In some embodiments, a serotype of the first pharmaceutical composition can be from the same bacteria as a serotype for the second pharmaceutical composition. For example, the first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F 38 and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and

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Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*,

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and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical compositions can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F,

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35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

[0090] Different serotypes of *S. pneumoniae* can be more pervasive or endemic in different geographical areas. For example, a certain group of serotypes can commonly cause pneumococcal disease in the United States and Canada. This group of serotypes can be different for Europe, the Middle East, North Africa, Japan and Korea. For example, administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Europe, the Middle East, and/or North Africa. Administration of a pharmaceutical composition to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 15C, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Japan and/or Korea. Additionally, invasive clinical disease can be caused by serotype 20B (as opposed to serotype 20A). The subject can be a human who resides in the specific geographical location as indicated above, or can be a human who is visiting the specific geographical location as indicated above.

[0091] A method of administration can comprise administering a first saccharide-polypeptide conjugate composition to a subject; administering a second saccharide-polypeptide conjugate composition to the subject at least four weeks after administering the first saccharide-

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polypeptide conjugate composition; wherein the first saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a second polypeptide of the second saccharide-polypeptide conjugate composition; and wherein the first saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. In some embodiments, the first serotype is from the same bacteria as the second serotype. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition comprises a saccharide-polypeptide conjugate comprising a polypeptide that has at less than about 70% length homology, about 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0092] In other embodiments, the conducting of any of the methods described herein may not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

Saccharide-polypeptide Conjugate Kits

[0093] The saccharide-polypeptide conjugates and pharmaceutical compositions described herein can be contained in a kit. The kit can further comprise instructions for use. The saccharide-polypeptide conjugates and/or pharmaceutical compositions can be stored in a container. The container can be a syringe. The container can be a vial. The vial can be a 2 mL borosilicate glass vial. The vial can be 3 mL Vials USP Type I glass, non-siliconized. The top can be an aluminum crimp top. The container can be a vial with a rubber septum or a septum made of flexible, reclose-able material. The container can be a vial with a stopper. The stopper can be a non-siliconized stopper. The vial can have a fill volume of 0.6 mL. The extractable volume can be 0.5 mL.

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EXAMPLES

[0094] The following examples are included to further describe some aspects of the present disclosure, and should not be used to limit the scope of the embodiments described herein.

EXAMPLE 1

Method of Establishing a Cell Bank

[0095] This example shows the establishment of a cell bank for a *S. pneumoniae* strain for use in producing polysaccharides for a polysaccharide-CRM_{197} conjugate vaccine. Any strain that produces the polysaccharides of **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are established as a cell bank by this method. A *S. pneumoniae* strain is provided by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG). The cell bank process is depicted in **FIGURE 2**. A vial of a strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol and then is dispensed into cryogenic vials. The cell bank is contained in the cryogenic vials, which are stored at -70°C until use.

[0096] *S. pneumoniae* strains that produce serotype 6C polysaccharide was acquired by the University of Alabama, Birmingham (UAB) for establishing a 6C cell bank. The cell bank process is depicted in **FIGURE 2**. For 6C polysaccharide serotype, a vial of the strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol and then is dispensed into cryogenic vials. The cell bank for each strain are contained in the cryogenic vials, which are stored at -70°C until use.

[0097] *S. pneumoniae* strains that produce serotype 8 polysaccharide, serotype 10A polysaccharide, serotype 11A polysaccharide, serotype 12F polysaccharide, serotype 22F

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polysaccharide, or serotype 33F polysaccharide were acquired by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG) for establishing a cell bank. The cell bank process is depicted in **FIGURE 2**. For each polysaccharide serotype, a vial of the strain of *S. pneumoniae* was seeded and then was grown overnight on an agar plate. A single colony was picked from this plate and streaked onto a new agar plate for overnight growth. This process was repeated three times to purify the strain before a flask was seeded and was incubated. After allowing the seeded flask sufficient time for growth, the flask contents were harvested by centrifugation. The centrifugation product was resuspended in a medium of about 20% glycerol and then was dispensed into cryogenic vials. The cell bank for each strain were contained in the cryogenic vials, which were stored at -70°C until use.

EXAMPLE 2

Method of Isolating and Purifying Polysaccharides

[0098] This examples shows how polysaccharides are isolated and purified from cell banks for use in a polysaccharide-CRM_{197} conjugate vaccine. Any of the polysaccharides of **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are isolated and purified by this method. This process for the isolation and purification of polysaccharides is shown in **FIGURE 4**. A vial of a cell bank produced as described in **EXAMPLE 1** is used to inoculate a flask containing an appropriate culture medium and incubated overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 μm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

[0099] A cell bank of an *S. pneumoniae* strain that produces serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F was used to inoculate a flask containing the appropriate culture medium and incubated overnight. This culture was used to seed a new culture for fermentation in a fermenter. The culture was allowed to grow under

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the appropriate conditions for the specific strain. After fermentation, the cells were inactivated and then were treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris was removed by centrifugation, which was followed by a depth filter step through a 0.45 μm filter and the cell free broth was collected. The product was then purified and the polysaccharide size was determined. Quality control tests were performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

EXAMPLE 3

Method of CRM_{197} production

[0100] This example shows the production of CRM_{197} (SEQ ID NO:1) for use in saccharide polypeptide vaccines, which is depicted in **FIGURE 3.** A vial from a working cell bank of CRM_{197} was seeded into a flask containing the appropriate culture medium and incubated overnight. The fermentation culture was centrifuged and the contents were harvested, were clarified, and then were filtered through a 0.2 μm filter. The filtrate was concentrated about ten-fold, and then was diafiltered to exchange the buffer and remove contaminants. The protein was further purified by microfiltration using an appropriate pore size membrane. Then the filtrate was further purified by anion exchange chromatography, followed by ammonium sulphate precipitation, and filtration (0.45 μm). A final purification using hydrophobic chromatography was conducted, and the highly purified CRM_{197} protein was concentrated and diafiltered, and then filtered through a 0.2 μm filter. The product was stored at -70°C until use in conjugation with a polysaccharide, such as any polysaccharide listed in **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)). Quality control tests were performed on the product to assess concentration (BCA for Lowry NLT 15 mg/mL), purity (SDS-PAGE gel with a target of ≥ 95% pure), confirming CRM_{197} identification (using a monoclonal antibody), assessing size (SDS-Page with a target of ~58 kDa), assessing endotoxin level (LAL kit with target of <1 IU/μg of protein), assessing lack of toxicity (Vero Cytotoxicity or DT enzyme test; non-lethal in Guinea Pigs), assessing pH (meter, target of 6.5 ± 0.2), and assessing appearance (visually, target for clear liquid).

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EXAMPLE 4

Method of conjugation

[0101] This example shows the conjugation of a capsular polysaccharide to a polypeptide via CDAP conjugation chemistry. The polysaccharide are isolated as described in **EXAMPLE 2** and are thawed. The polypeptide is chosen from any of the following: diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof. The polysaccharide is activated via CDAP chemistry in a CDAP solution. The polypeptide is added to the activated polysaccharides, and then the conjugation reaction is quenched. The reaction mixture is filtered, is diafiltrated, and is concentrated. The polysaccharide polypeptide conjugates are then separated using gel filtration chromatography. These conjugates are then concentrated for the final polysaccharide polypeptide conjugate.

EXAMPLE 5

Method of Polysaccharide-CRM_{197} Conjugation

[0102] This example shows the conjugation of a capsular polysaccharide to CRM_{197} via CDAP conjugation chemistry. The isolated polysaccharide is any polysaccharide as described in **TABLE 1** or of **FIGURES 11A-11F** (i.e., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)), or in **EXAMPLE 2**, and CRM_{197} is as described in **EXAMPLE 3**. The conjugation process is depicted in **FIGURE 5**. The polysaccharide and CRM_{197} are thawed. The polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} is added to the activated polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the

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polysaccharide-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The product is stored at 2-8°C.

[0103] The isolated polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F as described in **TABLE 1** and **EXAMPLE 2**, and CRM_{197} as described in **EXAMPLE 3** were thawed. The polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F were mixed with CDAP solution, which was then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} was added to the activated polysaccharides. The reaction was then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture was filtered, was diafiltered, and was concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates were then purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates were pooled, were concentrated, and then were filtered through a 0.2 μm filter. The product is stored at 2-8°C.

EXAMPLE 6

Combinations of Polysaccharide-CRM_{197} Conjugates in a Vaccine Composition

[0104] This example shows combinations of polysaccharides that are used in a polysaccharide-CRM_{197} conjugate vaccine. Each polysaccharide serotype is individually conjugated to a CRM_{197} to produce the polysaccharide CRM197 conjugate. The polysaccharide-CRM_{197} conjugates for each serotype are combined into vaccine composition. See the below **TABLE 2** for exemplary polysaccharide-CRM_{197} conjugate serotype combinations.

TABLE 2. Combinations of Polysaccharides in Polysaccharide-CRM_{197} Conjugate Vaccines

List of Polysaccharide Serotype Combinations
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33B, and 33F
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B

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List of Polysaccharide Serotype Combinations
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33B, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33B, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 23A, 23B, 31, 33A, 34, 35F, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 31, 33F, 33A, 34, 35B, 35F, and 38
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 20B, and 23F
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 20B,22F, 23A, 23B, 33B, and 33F
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B

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List of Polysaccharide Serotype Combinations
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 20B, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 20B, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, and 33B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33B, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 20B, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33B, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 31, 33A, 34, 35F, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 20B,

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List of Polysaccharide Serotype Combinations
22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 31, 33F, 33A, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 33A, 34, 35B, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B
6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B

EXAMPLE 7

Formulation of Polysaccharide-CRM_{197} Conjugate Vaccine

[0105] This example shows a formulation for a polysaccharide-CRM_{197} conjugate vaccine. Any combination of polysaccharide-CRM_{197} conjugates from **EXAMPLE 5** or the combinations listed in **EXAMPLE 6** are used in the vaccine composition. In addition to the polysaccharide-CRM_{197} conjugates, the vaccine is composed of normal saline and 0.01% Polysorbate 80, with a pH of 6.5 ± 0.2. This formulation of the polysaccharide CRM_{197} vaccine composition is stored in 2 mL glass vials with an aluminum crimp top and stopper at 2-8°C. The vial is filled to a volume of 0.60 mL to allow for an extractable volume of 0.50 mL.

EXAMPLE 8

Process for Formulating a Polysaccharide-CRM_{197} Conjugate Vaccine

[0106] This example shows the formulating process for a polysaccharide CRM_{197} conjugate vaccine. The polysaccharide CRM_{197} conjugates are prepared as described in the above Examples. The formulation process is depicted in **FIGURE 6**. 0.9% w/v sterile saline is added to a formulation container. Each type of conjugate is separately sterilized and the appropriate volume of each monovalent bulk polysaccharide-CRM_{197} conjugate is added

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(volume is based on the polysaccharide concentration) to the formulation container one at a time. The pH is adjusted to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH. Optionally, an adjuvant is added to the polysaccharide-CRM_{197} conjugate mixture, such as aluminum phosphate to 1 mg/mL final concentration. A calculated volume of sterile saline is added to reach the appropriate final concentration, and then the mixture is blended for approximately two hours. The formulate in the bulk container is then attached to the filling machine, which fills, stoppers, and seals the vials of the final vaccine product. These vial then are inspected, labeled, and packed for distribution. During this process, the pH, appearance of the vaccine, and the vaccine sterility is monitored. At the filling, stoppering, and sealing step, the appearance, pH, identity of polysaccharides, identity of CRM_{197}, sterility, saccharide content, total protein content, endotoxin content, pyrogen content, general safety, osmolality, and extractable volume are assessed. 1-5 microgram of polysaccharide per each serotype CRM_{197} conjugate is contained in a 0.5 mL of vaccine in a vial.

EXAMPLE 9

Rabbit Toxicology Safety Study

[0107] This example shows the rabbit toxicology studies that are used to assess vaccine safety. The polysaccharide polypeptide conjugate is produced according to the above **EXAMPLES 1**, **2**, **3**, and **5**, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide-CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly on days 1, 22, 43, 64, and 85 to rabbits, 5 months of age, (10/gender) at a dose of 0.5 ml/injection into the right thigh muscle. 0.5 ml 0.9% sodium chloride is injected in the Control groups (10/gender). 0.125 mg aluminum phosphate as an adjuvant is injected in the AlP04 group (10/gender). A 4 week dose free recovery period is undertaken in five (5) animals/gender/group after the last injection. Treatment-related mortality and any other toxicologically relevant changes in relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight is assessed to support lack of systemic toxicity. Injection site are

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analyzed for reactions and any chronic inflammation. Specific serum antibodies to each of the polysaccharides in the polysaccharide CRM_{197} conjugate vaccine is found in the test group.

[0108] The polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly with and without an aluminum phosphate adjuvant as a single 0.5 mL injection into the left thigh muscle of 3 male rabbits/group, 5 months of age. 0.5 ml 0.9% sodium chloride is administered to the control group (3 males). Treatment related mortality, changes in body weight, relative food consumption, or treatment related macroscopic or microscopic lesions is assessed.

EXAMPLE 10

Composition and Method of Administration of a Vaccine

[0109] This example shows a vaccine composition and a method of administration of that vaccine composition. An aqueous vaccine composition is composed of an immunogenic polysaccharide polypeptide conjugate comprising capsular polysaccharides from any serotype from **TABLE 1** conjugated to any one of diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof; or any combination of polysaccharide polypeptide conjugates as described in Example 6. The aqueous vaccine is formulated to contain from 1-4 microgram of each serotype of polysaccharide per polysaccharide polypeptide conjugate, aluminum phosphate (1 mg/mL), and 0.01% polysorbate 80 in a single 0.5 mL injection delivered intramuscularly to a subject. This vaccine is administered to subject, wherein the subject is a child of 4 weeks to 2 years old. This vaccine is administered to subject, wherein the subject is a child of 2 to 18 years

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old. This vaccine is administered to a subject, wherein the subject is an adult of at least 50 years of age.

EXAMPLE 11

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United States and Canada

[0110] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United States and/or Canada. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), such as serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United States and Canada is improved.

EXAMPLE 12

Vaccination for Protection against Endemic *S. pneumoniae* Serotypes in Europe, the Middle East, and North Africa

[0111] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Europe, the Middle East, and/or North Africa. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38, such as serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35F. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Europe, the Middle East, and North Africa is improved.

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EXAMPLE 13

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in Japan and Korea

[0112] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Japan and/or Korea. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38, such as serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Japan and Korea is improved.

EXAMPLE 14

Method of Making a 6C-CRM_{197} Conjugate Pharmaceutical Composition

[0113] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 6C serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 6C polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 6C polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 6C polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 6C-CRM_{197} conjugates. The 6C-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 6C-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 6C-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 15

Method of Making a 20B-CRM_{197} Conjugate Pharmaceutical Composition

[0114] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 20B serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 20B polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 20B

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polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 20B polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 20B-CRM_{197} conjugates. The 20B-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 20B-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 20B-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 16

Composition of a Kit with a Saccharide-Polypeptide Conjugate

[0115] This example shows the composition of a kit with a saccharide-polypeptide conjugate. A saccharide-polypeptide conjugate in a vial or a pre-filled syringe is placed in a container. The container is further associated with instructions of use.

EXAMPLE 17

Vaccination Against *S. pneumoniae*

[0116] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0117] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 18

Vaccination Against *S. pneumoniae*

[0118] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an

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immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0119] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 19

Vaccination Against *S. pneumoniae*

[0120] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes of any of the combinations listed in **TABLE 2**. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0121] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 20

Method of Vaccination

[0122] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an

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immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0123] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

EXAMPLE 21

Method of Vaccination

[0124] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0125] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

EXAMPLE 22

Method of Vaccination for an Adult Aged 50 Years Old or Older

[0126] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for an adult age 50 years old or older. A first aqueous vaccine composition is

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composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0127] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to an adult age 50 years old or older. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older.

EXAMPLE 23

Method of Vaccination for a Child Aged 2 to 18 Years Old

[0128] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 2 to 18 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0129] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 2 to 18 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old. Alternatively

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to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old.

EXAMPLE 24

Method of Vaccination for a Child Aged 4 Weeks to 2 Years Old

[0130] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 4 weeks to 2 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0131] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 4 weeks to 2 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child who is 2-18 years old. Alternatively to four weeks later, one year later a single 0.5 of the second aqueous vaccine is given by intramuscular injection to the child aged 4 weeks to 2 years old.

EXAMPLE 25

Method of Vaccination for *S. pneumoniae* and *N. meningitidis*

[0132] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *N. meningitidis*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C capsular saccharide conjugated to CRM_{197}. Each vaccine is formulated for intramuscular injection.

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[0133] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine is given by intramuscular injection.

EXAMPLE 26

Method of Vaccination for *S. pneumoniae* and *S. enterica*

[0134] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *S. enterica*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *S. enterica* subspecies enterica serovar Typhi Vi-capsular saccharide conjugated to CRM_{197}. Each aqueous vaccine is formulated for intramuscular injection.

[0135] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

EXAMPLE 27

Method of Vaccination for *S. pneumoniae*, *N. meningitidis,* and *H. influenzae*

[0136] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae*, *N. meningitidis,* and *H. influenzae*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to a tetanus toxoid carrier protein. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C and group Y saccharide and *H. influenzae* type b saccharide individually conjugated a tetanus toxoid carrier protein. Each aqueous vaccine is formulated for intramuscular injection.

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[0137] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

[0138] While preferred embodiments of the present invention have been shown and described herein, it will be apparent to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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(54) Title: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

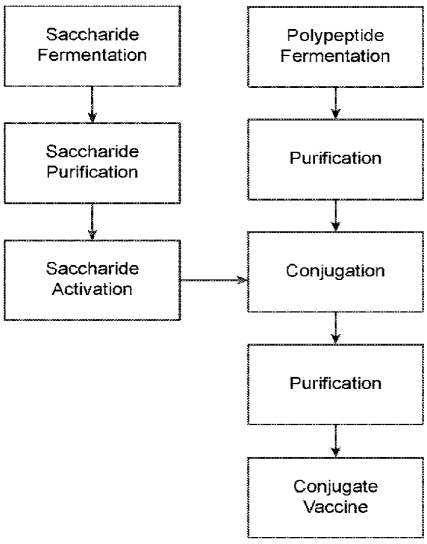


FIG. 1

[Continued on next page]

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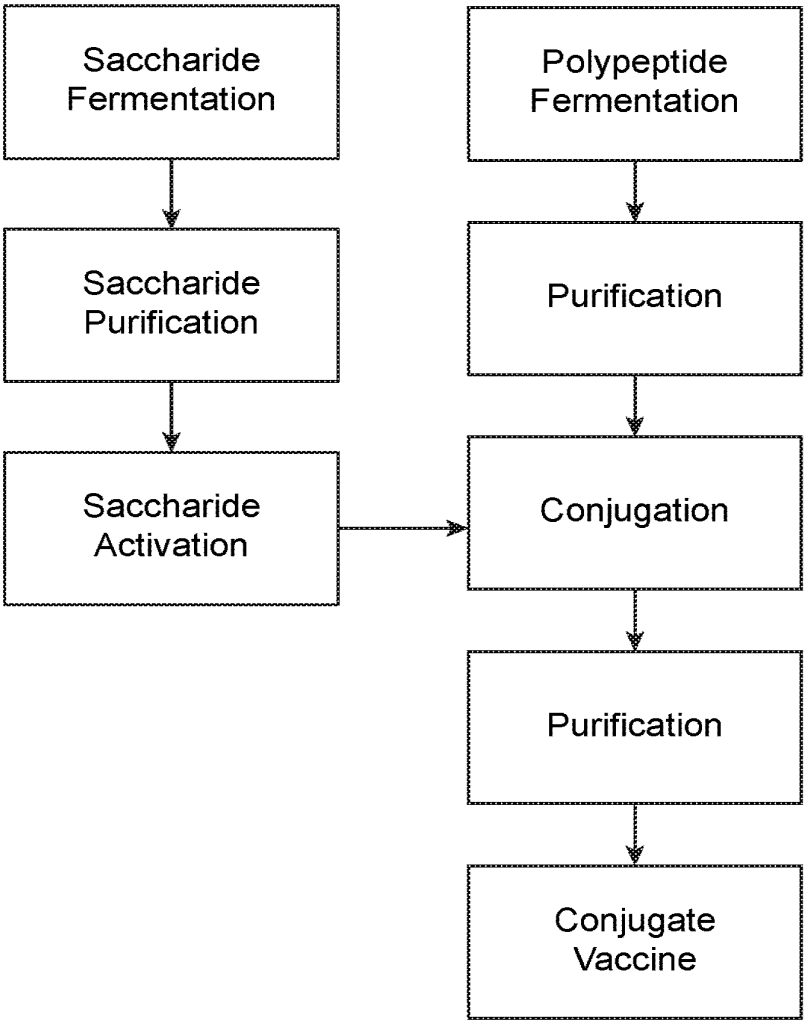


FIG. 1

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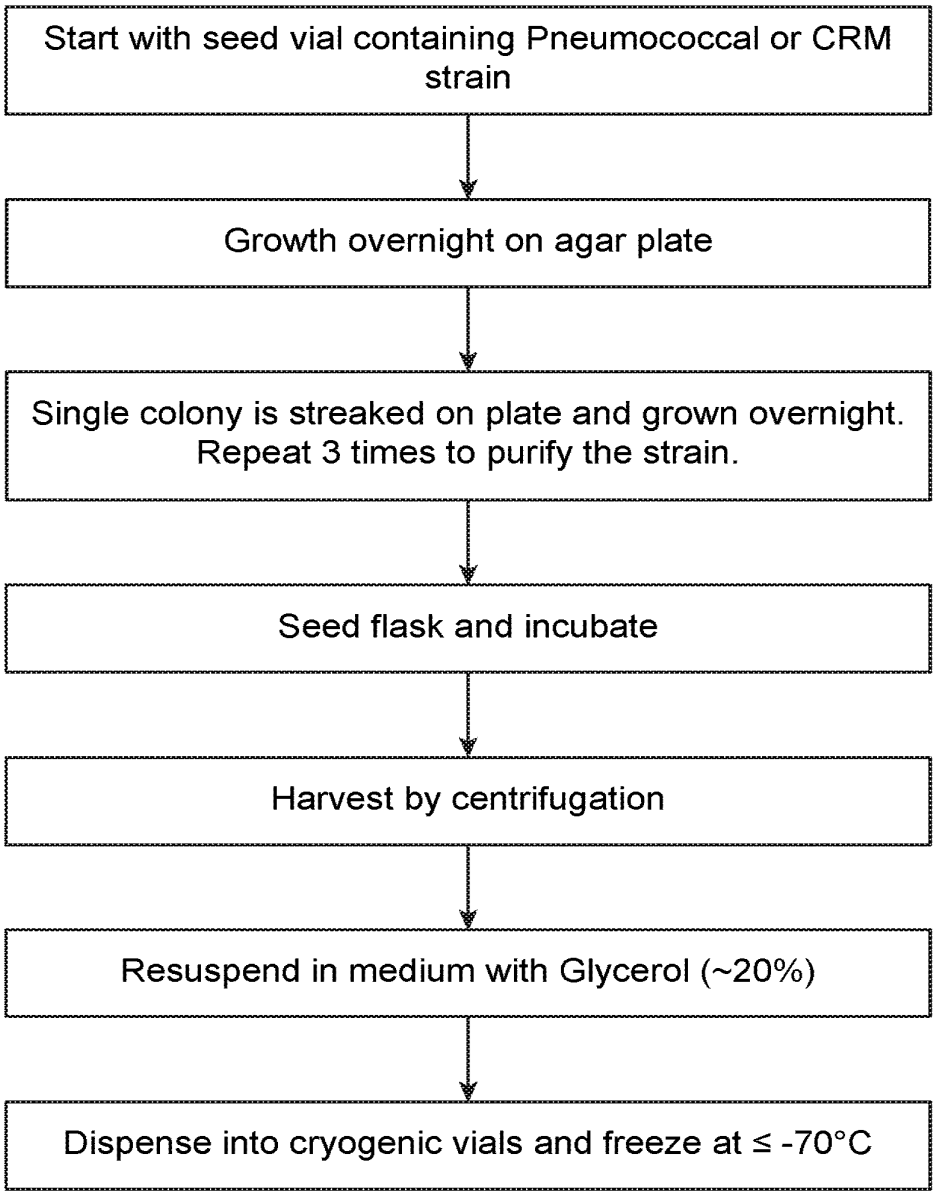


FIG. 2

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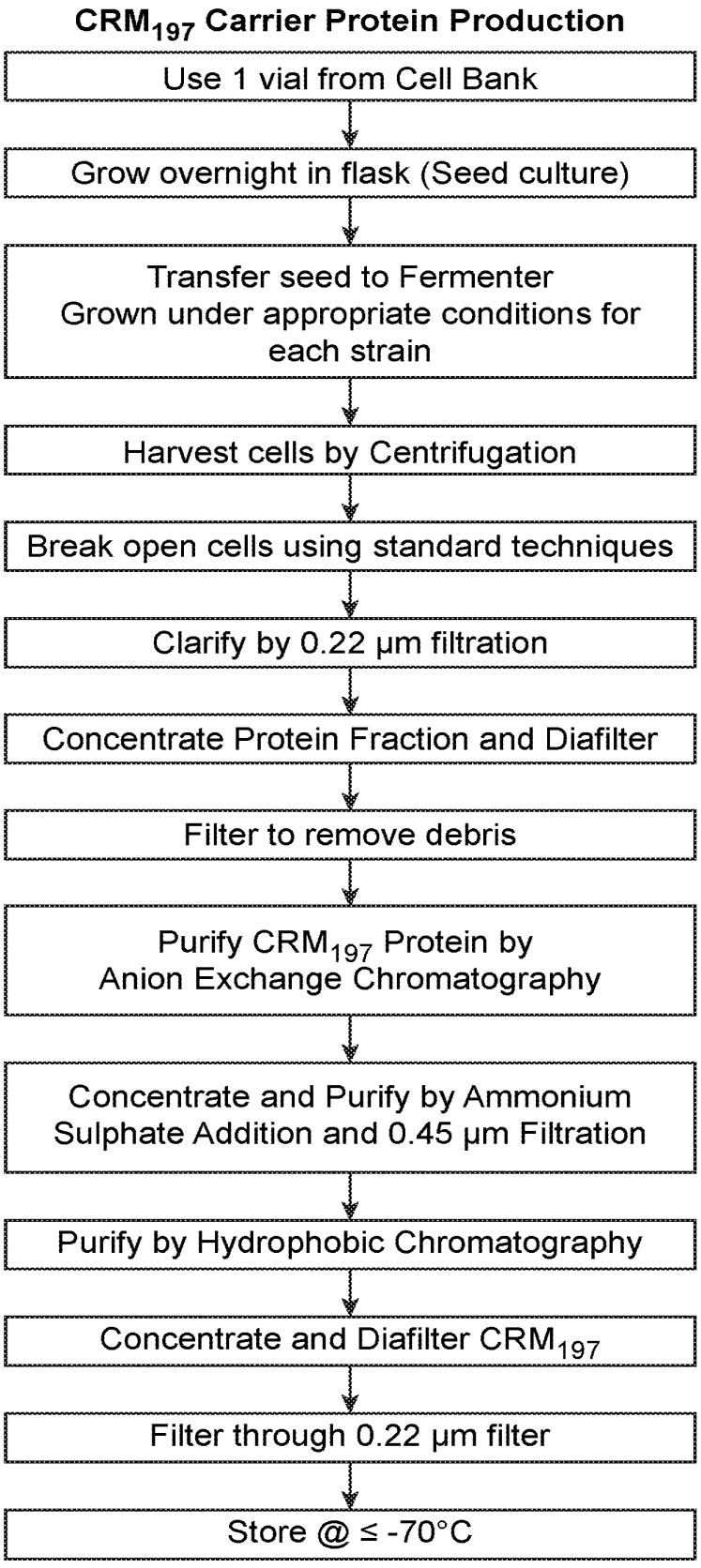


FIG. 3

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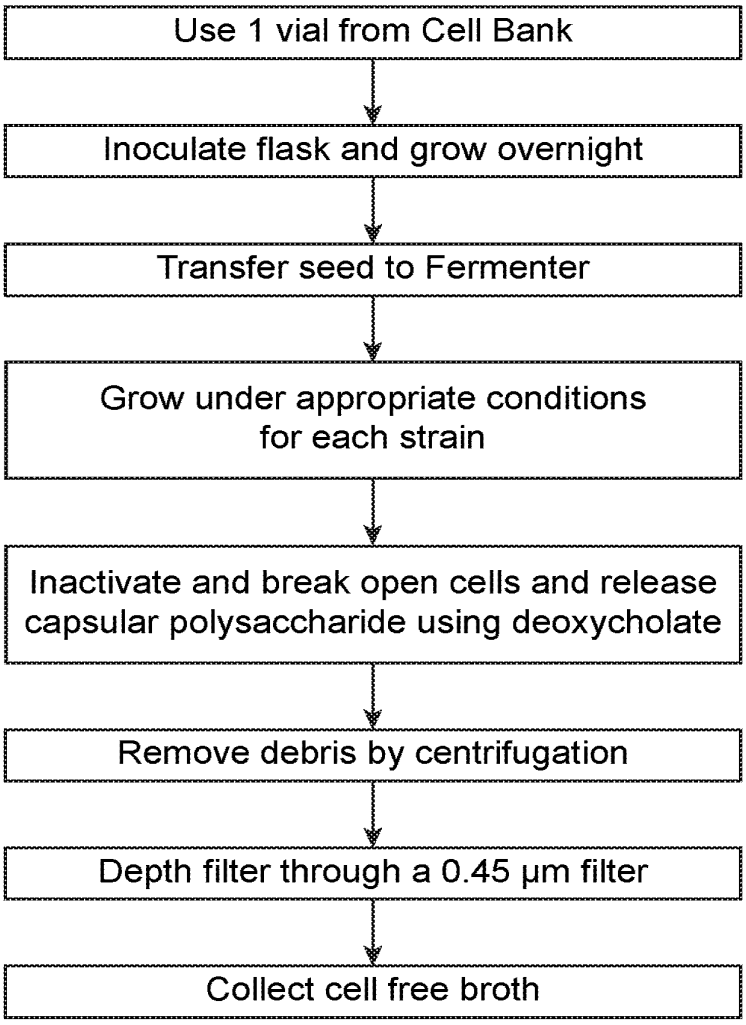


FIG. 4

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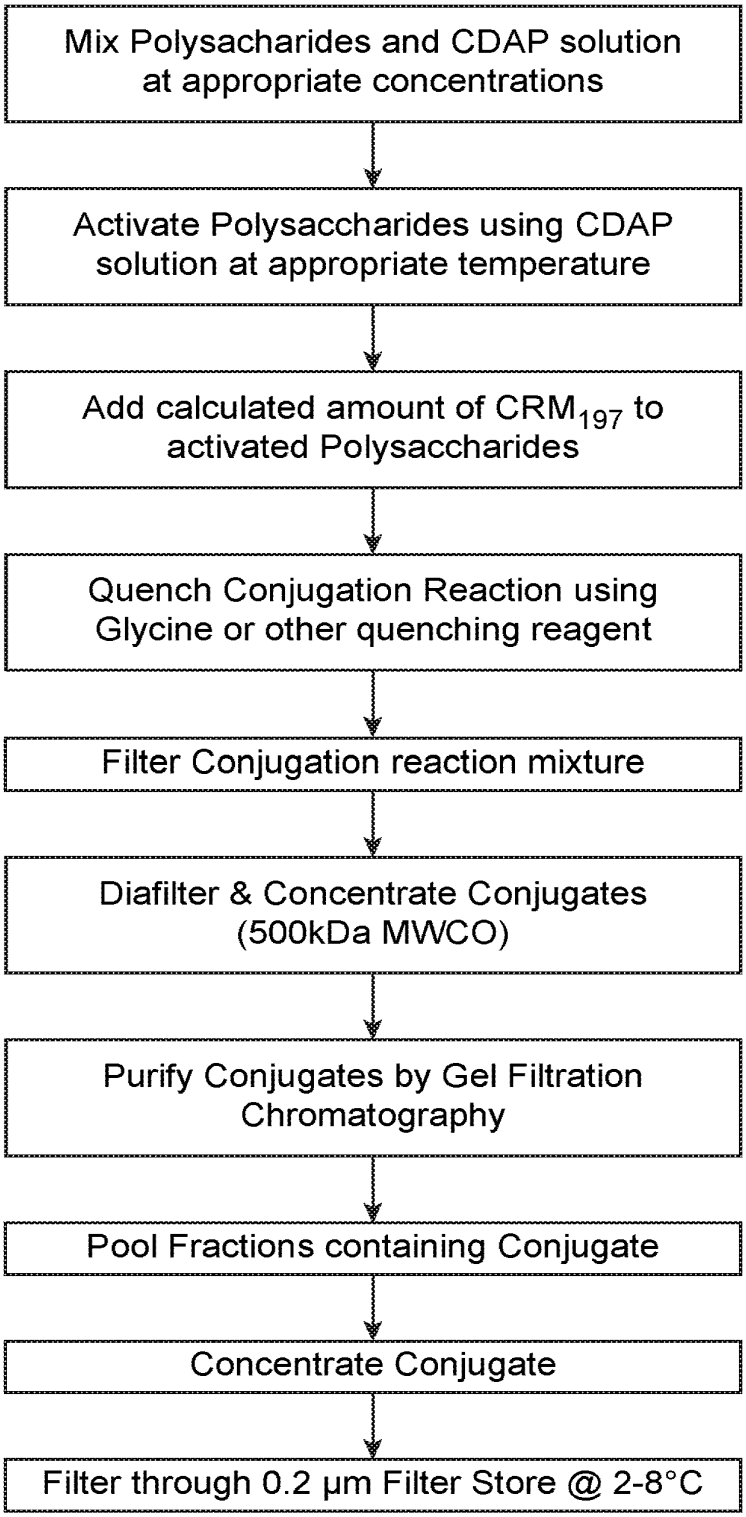


FIG. 5

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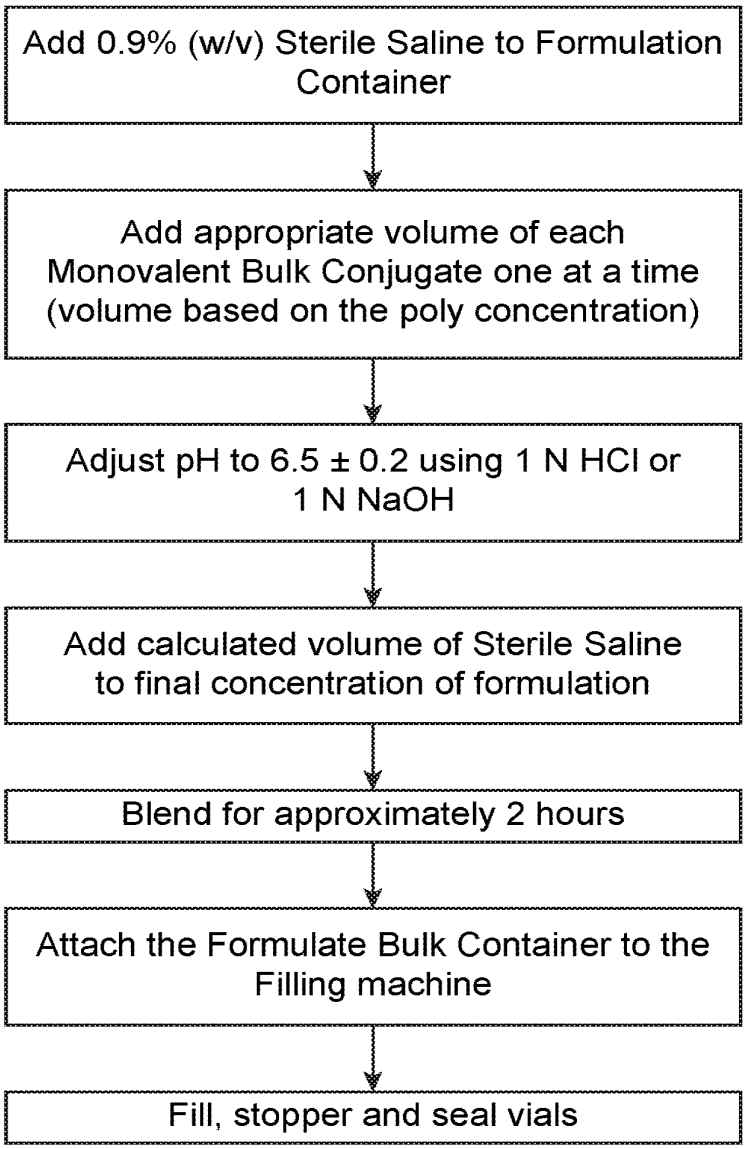


FIG. 6

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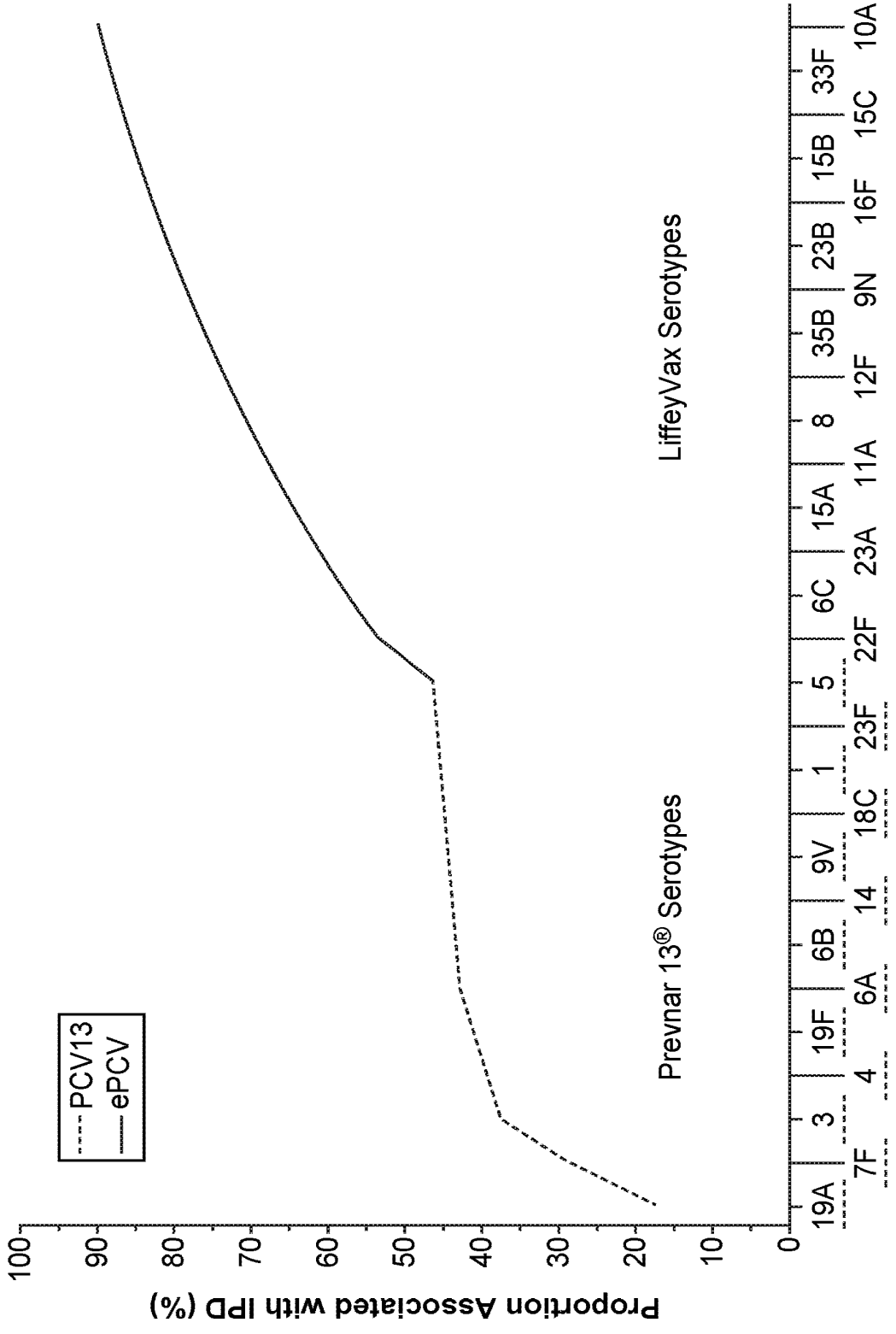


FIG. 7

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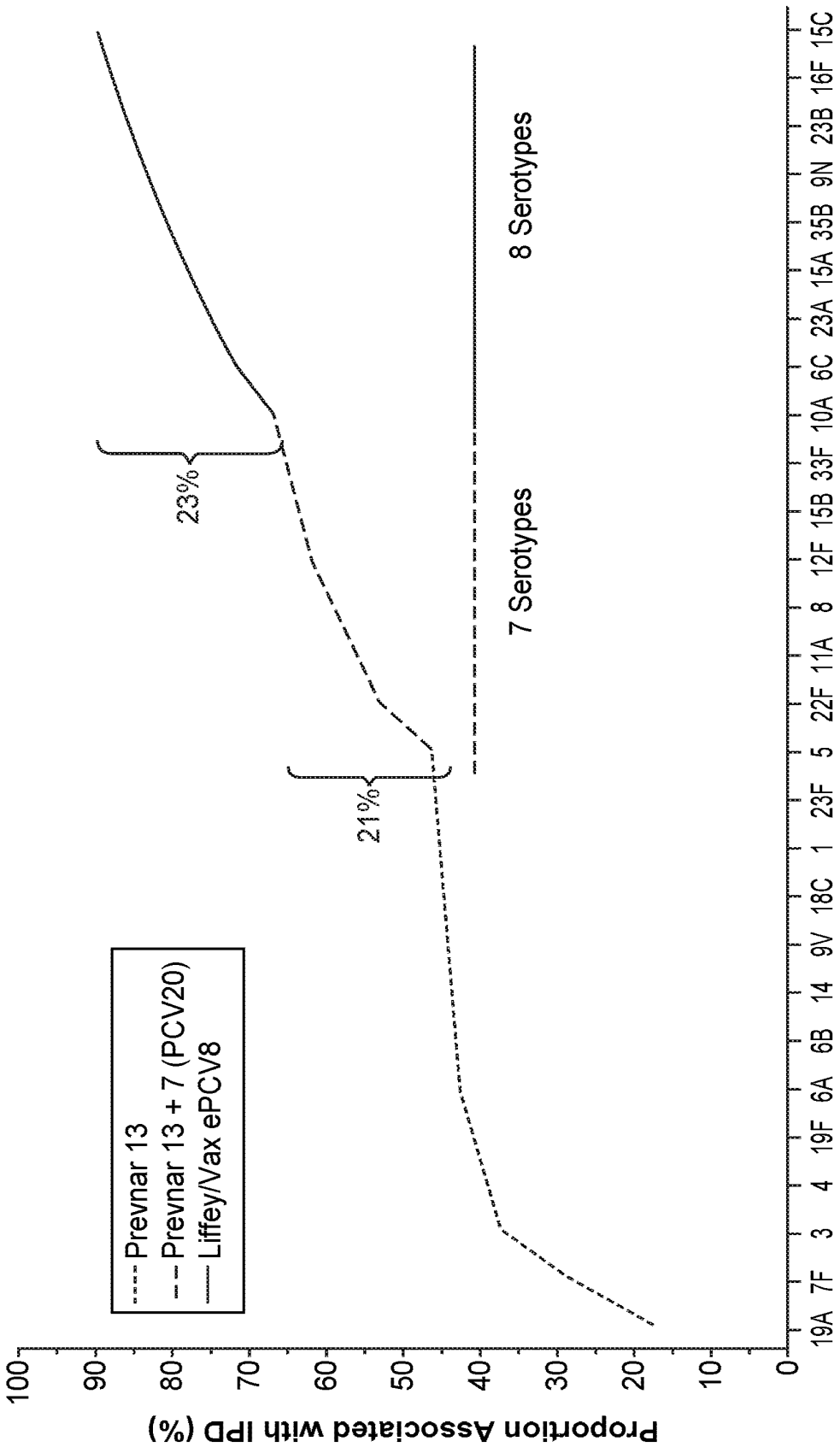


FIG. 8

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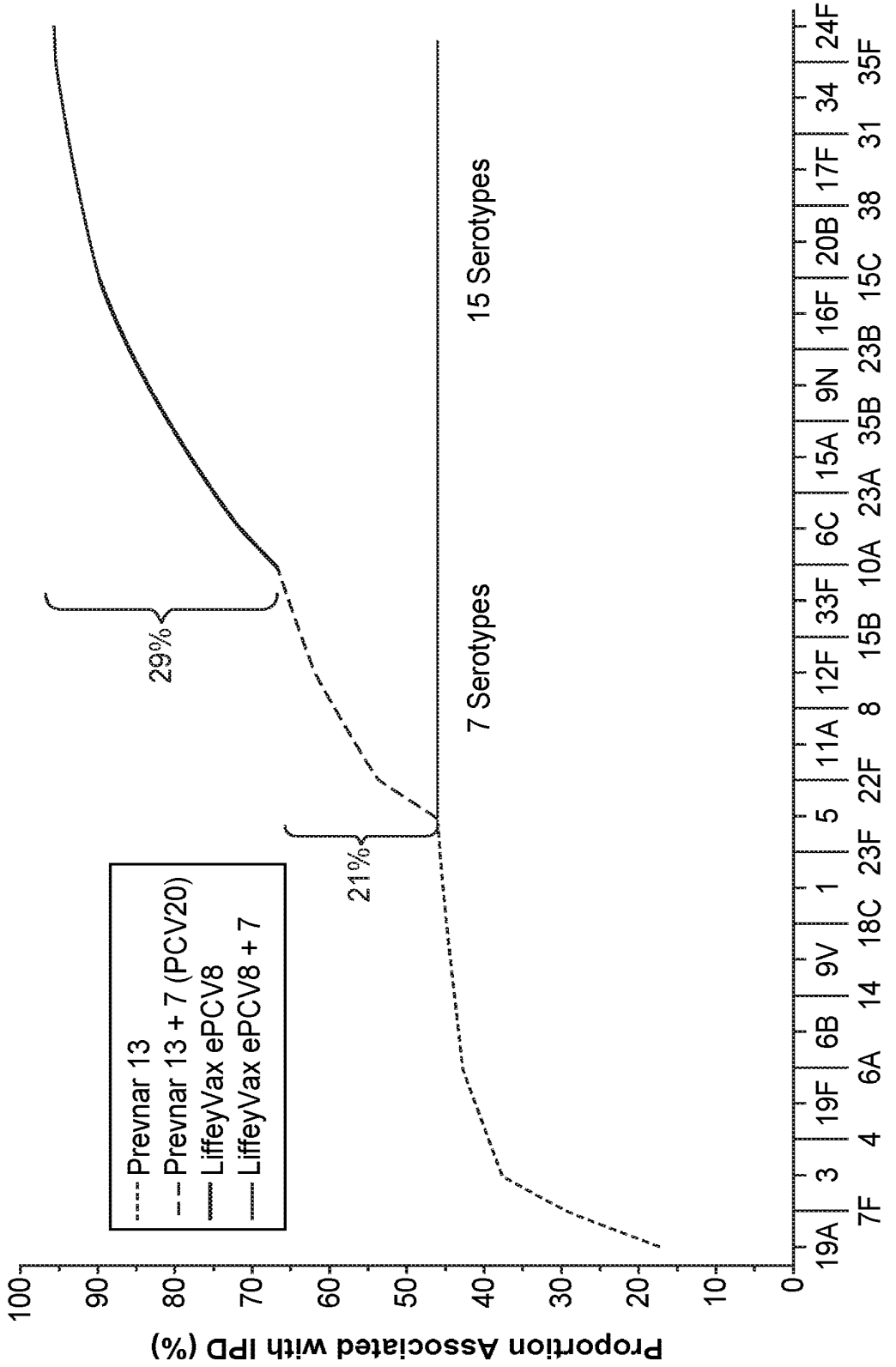


FIG. 9

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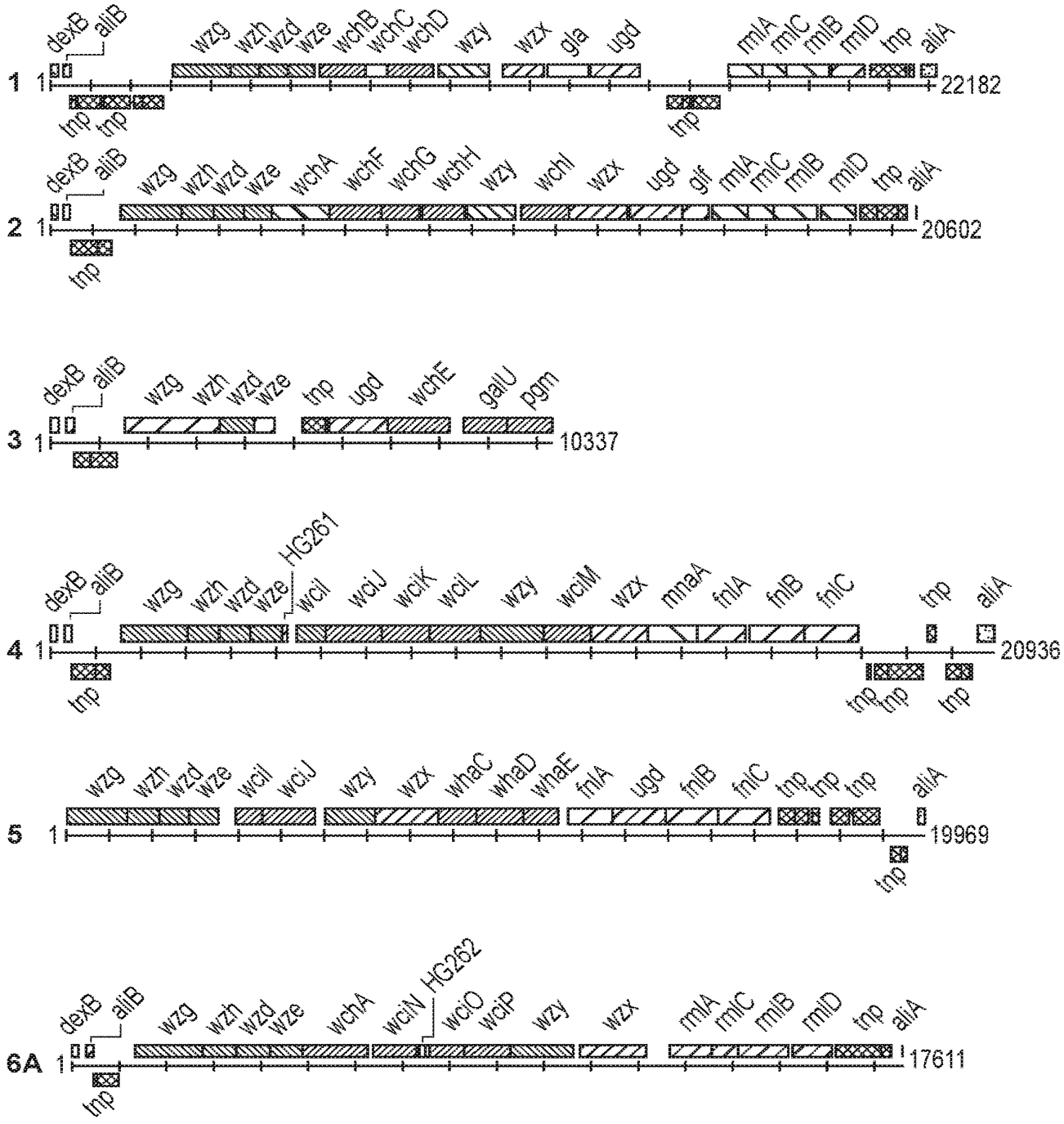


FIG. 10A

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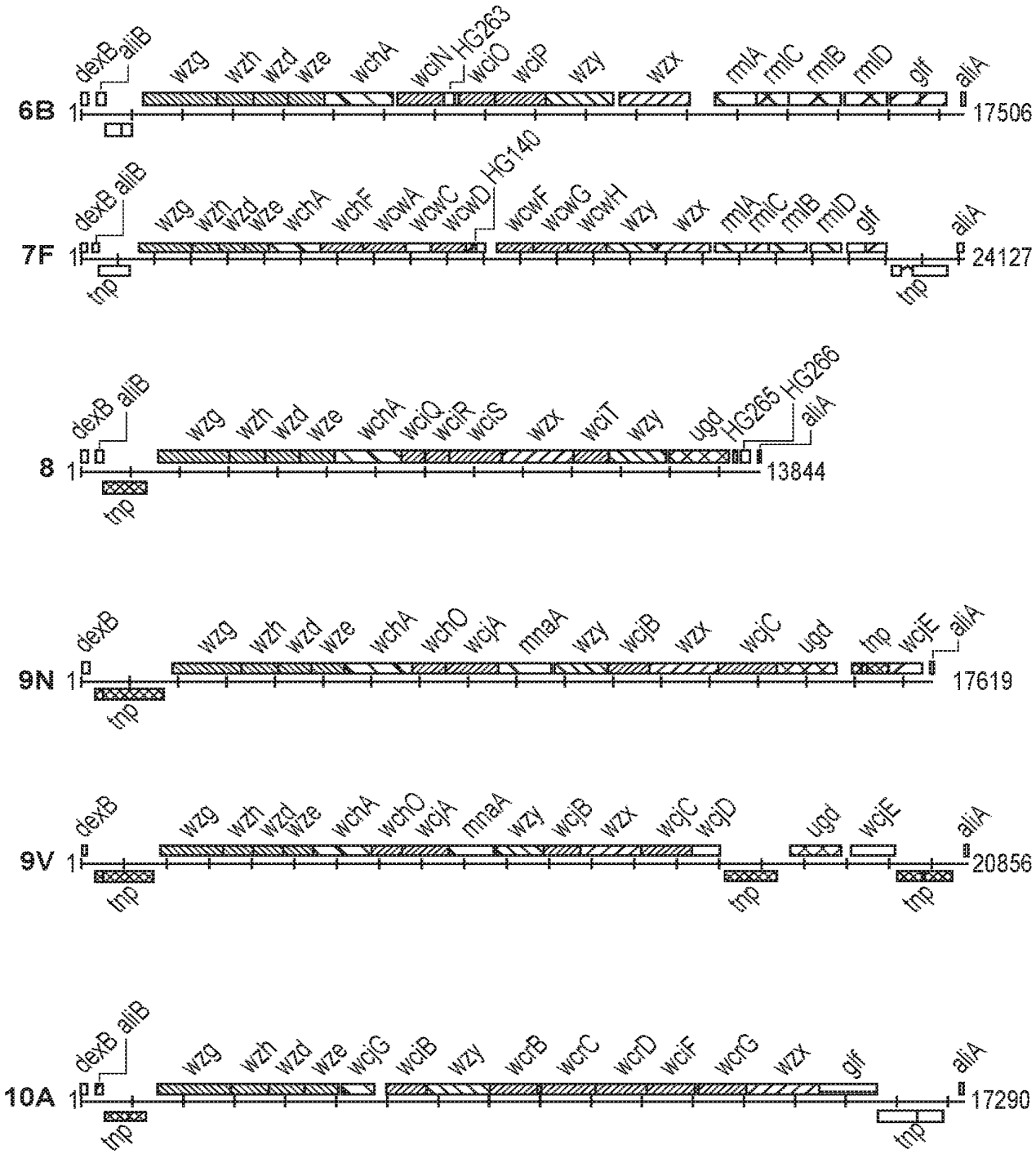


FIG. 10B

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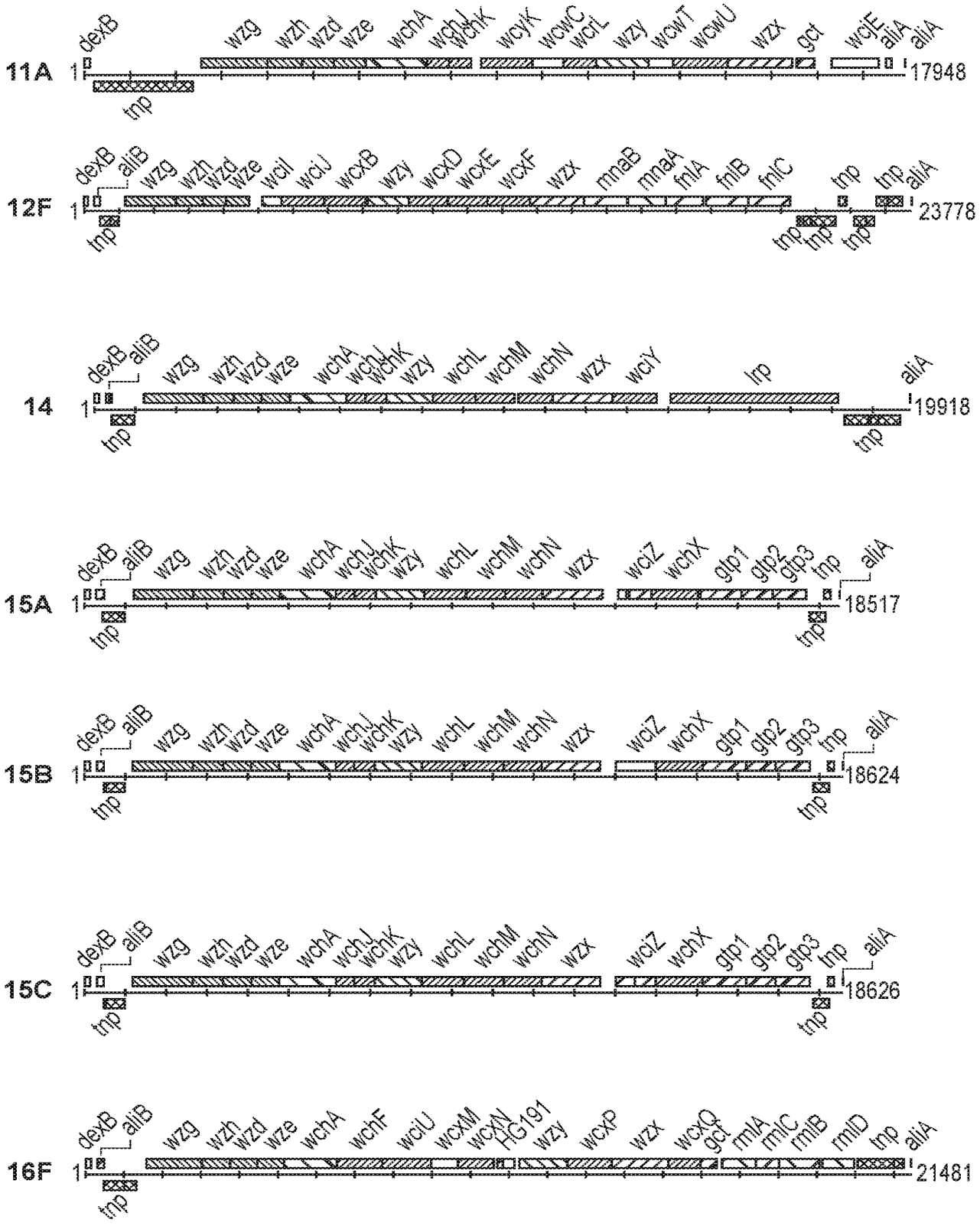


FIG. 10C

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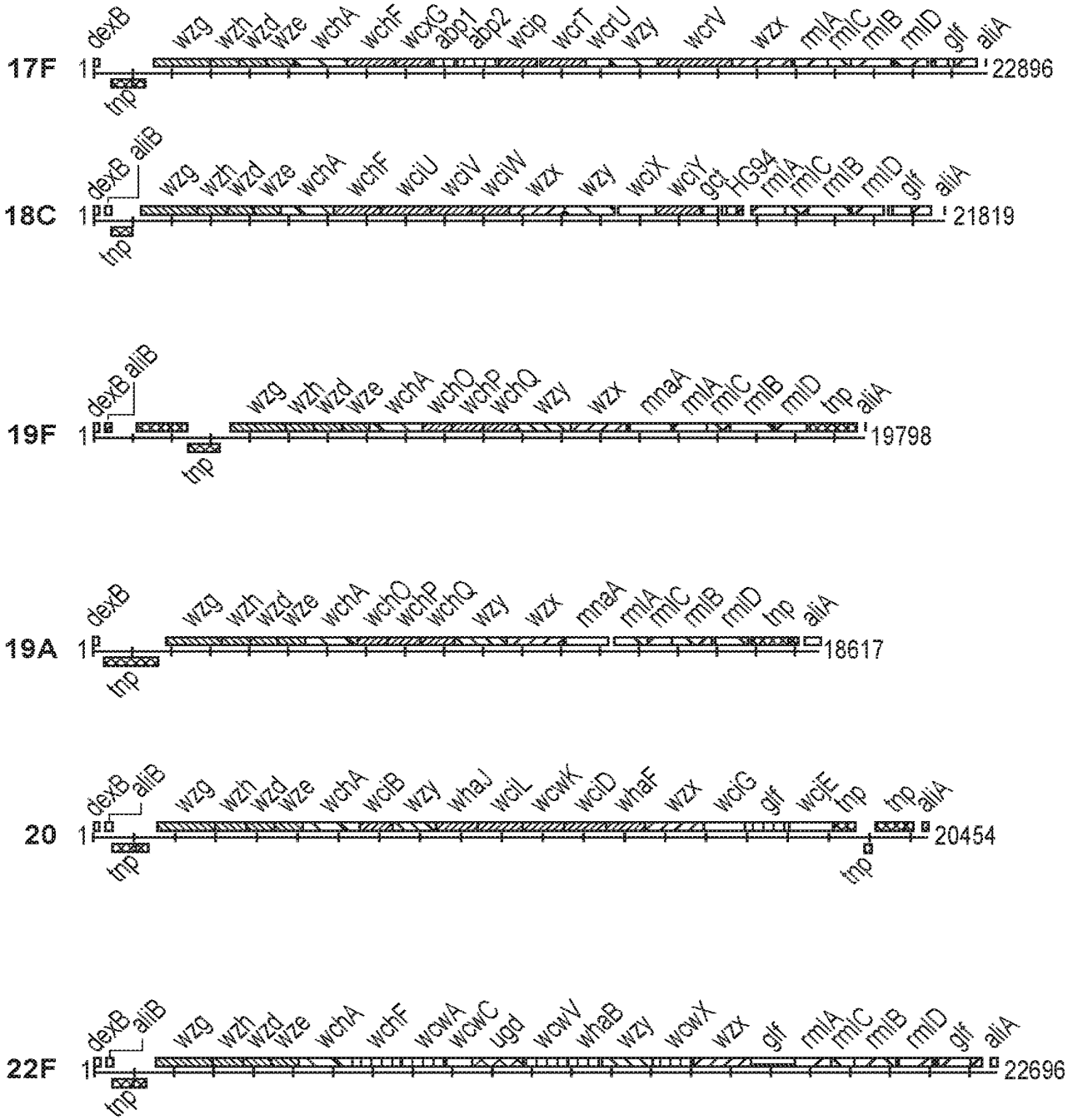


FIG. 10D

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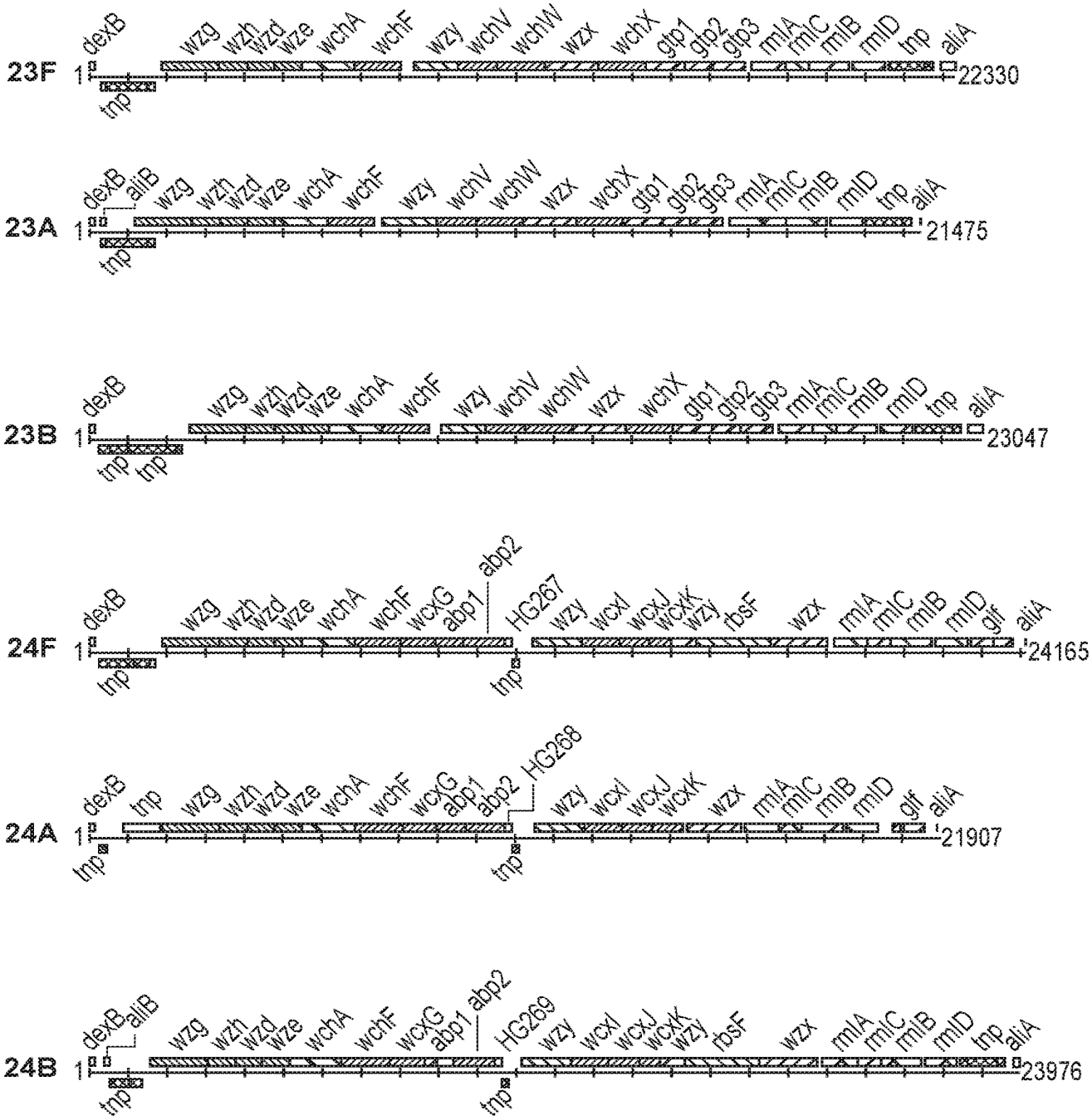


FIG. 10E

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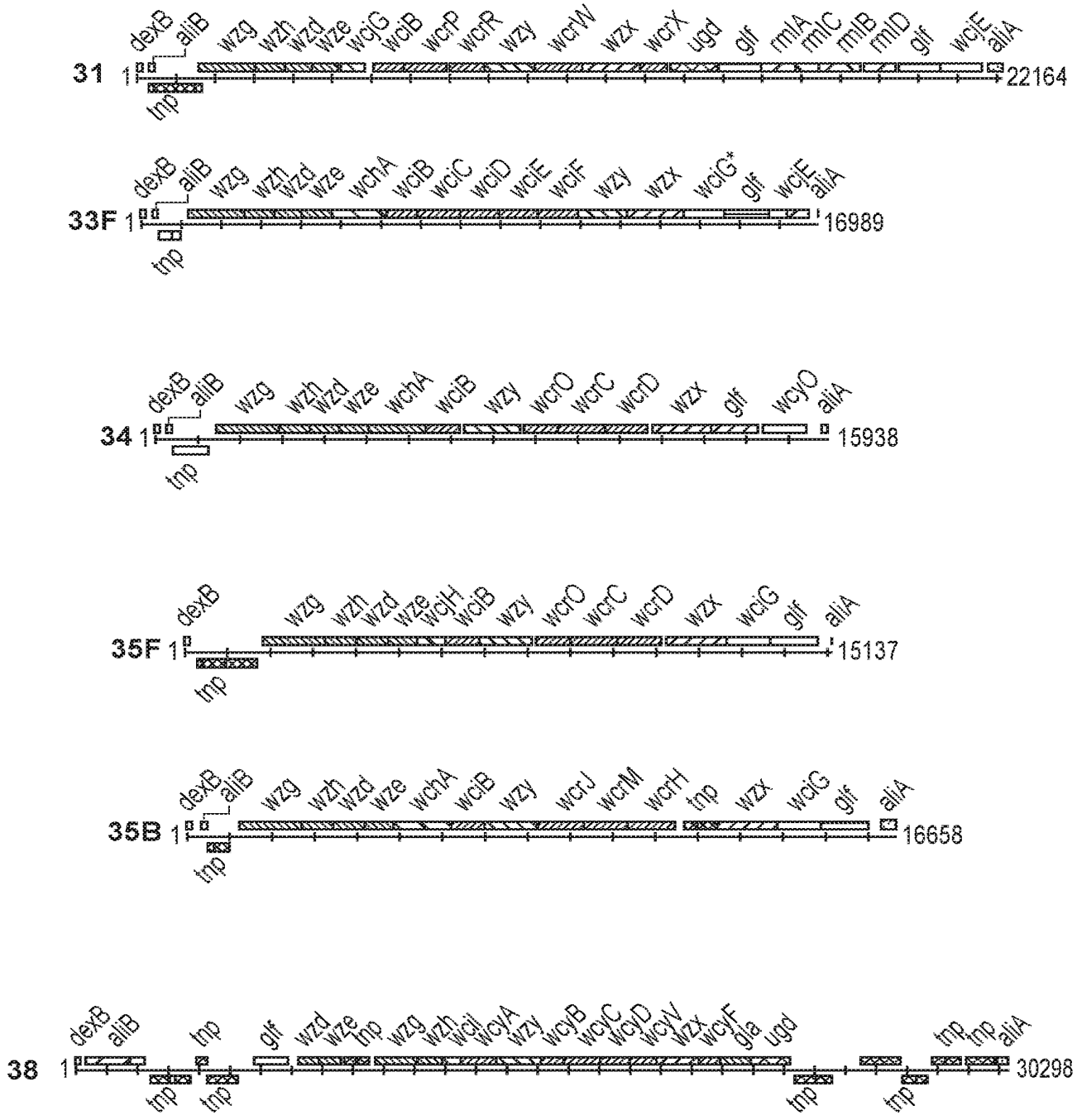


FIG. 10F

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CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, and 38.
2. The pharmaceutical composition of claim 1, comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.
3. The pharmaceutical composition of claim 1, comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.
4. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
5. The pharmaceutical composition of claim 1, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a

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unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

6. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
7. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.
8. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
9. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
10. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
11. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype

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selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

12. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
13. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
14. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
15. The pharmaceutical composition of claim 1, comprising a plurality of at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B.
16. The pharmaceutical composition of claim 1, comprising a plurality of at least 8 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
17. The pharmaceutical composition of claim 1, comprising a plurality of at least 7 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular

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polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 15A, 15C, 16F, 23A, 23B, and 33B.

18. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
19. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
20. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
21. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.
22. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.

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23. The pharmaceutical composition of any one of claims 1-22, wherein the unique *Streptococcus pneumoniae* serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
24. A pharmaceutical composition comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 34, 35B, and 38.
25. The pharmaceutical composition of claim 24, comprising a plurality of at least 15 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38.
26. The pharmaceutical composition of claim 24, comprising a plurality of at least 25 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.
27. The pharmaceutical composition of claim 24, comprising a plurality of at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B.
28. The pharmaceutical composition of claim 24, comprising a plurality of at least 19 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide,

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wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 24F, 35B and 38.

29. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.
30. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.
31. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
32. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B.
33. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
34. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.
35. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
36. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.

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37. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
38. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 23A, 23B, and 33B.
39. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
40. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
41. The pharmaceutical composition of claim 29, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
42. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
43. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
44. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
45. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.
46. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
47. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B.
48. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
49. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F.
50. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 20B, 23A, 23B, 33F, and 35B.

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51. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.
52. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 15A, 15C, 16F, 23A, 23B, and 33B.
53. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B.
54. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B, and 35B.
55. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33B, 34, 35F, 35B, 24F, and 31.
56. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33B, 35F, and 35B.
57. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, and 33B.
58. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of the group consisting of 15A, 15C, 16F, 23A, 23B, and 33B.
59. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
60. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
61. The pharmaceutical composition of claim 29, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
62. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 20B and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.
63. The pharmaceutical composition of claim 62, wherein the at least one additional serotype consists of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B.

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64. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
65. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B.
66. The pharmaceutical composition of claim 62, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33B, 35F, and 35B.
67. A pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof conjugated to a polypeptide, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, and 38.
68. The pharmaceutical composition of claim 67, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.
69. The pharmaceutical composition of claim 67, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
70. The pharmaceutical composition of claim 67, further comprising at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38.
71. The pharmaceutical composition of any one of claims 1-70, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

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72. The pharmaceutical composition of any one of claims 1-71, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.
73. The pharmaceutical composition of claim 72, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.
74. The pharmaceutical composition of any one of claims 1-73, wherein at least one polypeptide of the plurality is an oligopeptide.
75. The pharmaceutical composition of any one of claims 1-74, wherein at least one polypeptide of the plurality comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.
76. The pharmaceutical composition of any one of claims 1-75, wherein the polypeptide comprises a mixture of polypeptides.
77. The pharmaceutical composition of any one of claims 1-76, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.
78. The pharmaceutical composition of any one of claims 1-77, further comprising an adjuvant.
79. The pharmaceutical composition in unit dose form of claim 78, wherein the adjuvant is an aluminum-cation comprising adjuvant.
80. The pharmaceutical composition of claim 78, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.
81. The pharmaceutical composition of any one of claims 1-80, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.
82. The pharmaceutical composition of any one of claims 1-81, further comprising a chelating agent.

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83. The pharmaceutical composition of claim 82, wherein the chelating agent comprises ethylenediaminetetraacetic acid (EDTA).
84. The pharmaceutical composition of any one of claims 1-83, which is in a form of a suspension.
85. The pharmaceutical composition of any one of claims 1-84, which is in the form of a homogenous mixture or a heterogeneous mixture.
86. The pharmaceutical composition of claim 85, wherein the pharmaceutical composition is in a form of a homogenous mixture.
87. The pharmaceutical composition of any one of claims 1-86, which is contained in a pre-filled syringe.
88. The pharmaceutical composition of any one of claims 1-87, further comprising a surfactant , an emulsifier, or a combination thereof.
89. The pharmaceutical composition of claim 88, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.
90. The pharmaceutical composition of claim 89, comprising the polysorbate, wherein the polysorbate is polysorbate 80.
91. The pharmaceutical composition of claim 89, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.
92. The pharmaceutical composition of any one of claims 1-91, further comprising a buffering agent.
93. The pharmaceutical composition of claim 92, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.
94. The pharmaceutical composition of any one of claims 1-93, further comprising sterile water.
95. The pharmaceutical composition of any one of claims 1-94, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.
96. The pharmaceutical composition of any one of claims 1-95, wherein when the pharmaceutical composition is administered intramuscularly to a human, the

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pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

97. The pharmaceutical composition of any one of claims 1-96, wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

98. The pharmaceutical composition of any one of claims 1-97, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

99. The pharmaceutical composition of any one of claims 1-98, wherein the pharmaceutical composition is in unit dose form.

100. The pharmaceutical composition of any one of claims 1-99, further comprising a preservative.

101. The pharmaceutical composition of any one of claims 1-100, further comprising an anti-fungal compound or a salt thereof.

102. The pharmaceutical composition of any one of claims 1-101, further comprising a salt.

103. The pharmaceutical composition of any one of claims 1-102, wherein the salt is an inorganic salt.

104. The pharmaceutical composition of any one of claims 1-103, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

105. The pharmaceutical composition of any one of claims 1-104, which is a vaccine.

106. The pharmaceutical composition of any one of claims 1-105, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

107. The pharmaceutical composition of any one of claims 1-106, wherein the immunogenic saccharide-polypeptide conjugate is produced using bacteria.

108. The pharmaceutical composition of any one of claims 1-107, wherein the immunogenic saccharide-polypeptide conjugate is produced using *E. coli*.

109. The pharmaceutical composition of any one of claims 1-108, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

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110. The pharmaceutical composition of any one of claims 1-109, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

111. The pharmaceutical composition of any one of claims 1-110, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

112. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 1-111.

113. The method of claim 112, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising an immunogenic saccharide-polypeptide conjugate comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

114. The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

115. The method of any one of claims 113-114, wherein the second composition comprises a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

116. The method of any one of claims 113-115, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

117. The method of any one claims 113-116, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising each

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comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.

118. The method of claim 117, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.

119. The method of claim 118, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a fragment of the capsular polysaccharide.

120. The method of claim 119, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

121. The method of any one of claims 113-120, wherein the second polypeptide is an oligopeptide.

122. The method of any one of claims 113-121, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.

123. The method of any one of claims 113-122, wherein a toxin activity of the second polypeptide is at least partly mitigated.

124. The method of any one of claims 113-123, wherein the second composition further comprises an adjuvant.

125. The method of claim 124, wherein the adjuvant is an aluminum-cation comprising adjuvant.

126. The method of claim 125, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

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127. The method any one of claims 113-126, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

128. The method of any one of claims 113-127, wherein the second composition is in unit dose form.

129. The method of any one of claims 113-128, wherein the second composition further comprises a chelating agent.

130. The method of claim 129, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

131. The method any one of claims 113-130, wherein the second composition is in a form of a suspension.

132. The method any one of claims 113-131, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.

133. The method any one of claims 113-132, wherein the second composition is in the form of a homogenous mixture.

134. The method any one of claims 113-133, wherein the second composition is contained in a pre-filled syringe.

135. The method any one of claims 113-134, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.

136. The method claim 135, wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

137. The method of claim 136, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.

138. The method of claim 137, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

139. The method any one of claims 113-138, wherein the second composition further comprises a buffering agent.

140. The method of claim 139, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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141. The method any one of claims 113-140, wherein the second composition further comprises sterile water.

142. The method any one of claims 113-141, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

143. The method any one of claims 113-142, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

144. The method any one of claims 113-143, wherein when the unique immunogenic saccharide-polypeptide conjugate of the second composition elicits an opsonophagocytic response.

145. The method of any one of claims 113-144, wherein when the second composition is administered intramuscularly, the second composition elicits an immune response that lasts from about 1 week to about 30 years.

146. The method of any one of claims 113-145, wherein second composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

147. The method of any one of claims 113-146, wherein the second composition further comprises a preservative.

148. The method of any one of claims 113-147, wherein the second composition further comprises an anti-fungal compound or a salt thereof.

149. The method of any one of claims 113-148, wherein the second composition further comprises a salt.

150. The method of claims 149, wherein the salt is an inorganic salt.

151. The method of any one of claims 113-150, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is isolated and purified.

152. The method of any one of claims 113-151, wherein the second composition is a vaccine.

153. The method of any one of claims 113-152, which is a method of vaccinating a subject.

154. The method of claim 153, wherein the subject is a human.

155. The method of any one of claims 153-154, wherein the subject or human is a subject or human in need thereof.

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156. The method of any one of claims 113-155, wherein the immunogenic saccharide-polypeptide conjugates of the second composition are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

157. The method of any one of claims 113-156, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate of the second composition, wherein the antibody response is measured by measuring antibody titers.

158. The method of any one of claims 113-157, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using bacteria.

159. The method of any one of claims 113-158, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using *E. coli*.

160. The method of any one of claims 113-159, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

161. The method of any one of claims 113-160, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

162. The method of any one of claims 113-161, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

163. A method comprising:

administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition;

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and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

164. The method of claim 163, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

165. The method of claim 164, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

166. The method of claim 165, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

167. The method of any one of claims 163-166, wherein the first polypeptide is an oligopeptide.

168. The method of any one of claims 163-167, wherein the second polypeptide is an oligopeptide.

169. The method of any one of claims 163-168, wherein the first polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof .

170. The method of any one of claims 163-169, wherein a toxin activity of the first polypeptide is at least partly mitigated.

171. The method of any one of claims 163-170, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M. catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, any fragment thereof, or any combination thereof.

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172. The method of any one of claims 163-171, wherein a toxin activity of the second polypeptide is at least partly mitigated.

173. The method of any one of claims 163-172, wherein the first serotype is from a same bacteria as the second serotype.

174. The method of any one of claims 163-173, wherein the first serotype is from a different bacteria than the second serotype.

175. The method of any one of claims 163-174, wherein the first serotype is from *Streptococcus pneumoniae*.

176. The method of any one of claims 163-174, wherein the first serotype is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

177. The method of any one of claims 163-176, wherein the second serotype is from *Streptococcus pneumoniae*.

178. The method of any one of claims 163-176, wherein the second bacteria is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

179. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

180. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

181. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

182. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

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183. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*.

184. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*.

185. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*.

186. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*.

187. The method of any one of claims 163-178, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*.

188. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

189. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 15A, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

190. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

191. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus*

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pneumoniae, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

192. The method of any one of claims 163-178, wherein the first serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

193. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

194. The method of any one of claims 163-178, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

195. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

196. The method of any one of claims 163-178, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

197. The method of any one of claims 163-196, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

198. The method of claim 197, wherein the adjuvant is an aluminum-cation comprising adjuvant.

199. The method of claim 198, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

200. The method of any one of claims 163-199, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable

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composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

201. The method of any one of claims 163-200, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

202. The method of claim 201, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

203. The method of any one of claims 163-202, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

204. The method of any one of claims 163-203, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

205. The method of any one of claims 163-204, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

206. The method of any one of claims 163-205, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

207. The method of any one of claims 163-206, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

208. The method of claim 207, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

209. The method of claim 208, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

210. The method claim 208, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

211. The method of any one of claims 163-210, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

212. The method of claim 211, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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213. The method of any one of claims 163-212, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

214. The method of any one of claims 163-213, wherein when the first immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

215. The method of any one of claims 163-214, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

216. The method any one of claims 163-215, wherein when first immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

217. The method of any one of claims 163-216, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

218. The method of any one of claims 163-217, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

219. The method of claim 218, wherein the adjuvant is an aluminum-cation comprising adjuvant.

220. The method of claim 218, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

221. The method of any one of claims 163-220, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

222. The method of any one of claims 163-221, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

223. The method of claim 222, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

224. The method of any one of claims 163-223, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

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225. The method of any one of claims 163-224, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

226. The method of claim 225, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

227. The method of any one of claims 163-226, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

228. The method of any one of claims 163-227, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

229. The method of claim 228, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

230. The method of claim 229, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

231. The method of claim 230, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

232. The method of any one of claims 163-231, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

233. The method of claim 232, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

234. The method of any one of claims 163-233, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

235. The method of any one of claims 163-234, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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236. The method of any one of claims 163-235, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

237. The method of any one of claims 163-236, wherein when the second immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

238. The method of any one of claims 163-237, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

239. The method of any one of claims 163-238, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is in unit dose form.

240. The method of any one of claims 163-239, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises a preservative.

241. The method of any one of claims 163-240, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises an anti-fungal compound or a salt thereof.

242. The method of any one of claims 163-241, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprise a salt.

243. The method of claim 242, wherein the salt is an inorganic salt.

244. The method of any one of claims 163-243, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is isolated and purified.

245. The method of any one of claims 163-244, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition which is a vaccine.

246. The method of any one of claims 163-245, which is a method of vaccinating a subject.

247. The method of any claim 246, wherein the subject is a human.

248. The method of any one of claims 246-247, wherein the subject or human is a subject or human in need thereof.

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249. The method of any one of claims 163-248, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprise immunogenic saccharide-polypeptide conjugate(s) individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

250. The method of any one of claims 163-249, wherein administration of the second immunogenic saccharide-polypeptide conjugate composition does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

251. The method of any one of claims 163-250, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using a bacteria.

252. The method of any one of claims 163-251, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using *E. coli*.

253. The method of any one of claims 163-252, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

254. The method of any one of claims 163-253, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

255. The method of any one of claims 163-254, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

256. A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 1-70 with an excipient, an adjuvant, or any combination thereof.

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257. The method of claim 256, wherein the composition is injectable or intranasally administrable.

258. The method claim 256, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

259. The method of any one of claims 256-258, wherein the composition is in the form of an intramuscularly injectable composition.

260. The method of any one of claims 256-259, further comprising contacting the composition with a chelating agent.

261. The method of claim 260, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

262. The method of any one of claims 256-261, wherein the composition is in a form of a suspension.

263. The method of any one of claims 256-262, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

264. The method of any one of claims 263, wherein the composition is in a form of a homogenous mixture.

265. The method of any one of claims 256-264, wherein the composition is contained in a pre-filled syringe.

266. The method of any one of claims 256-265, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

267. The method of claim 266, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

268. The method of claim 267, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

269. The method of claim 268, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

270. The method of any one of claims 256-269, further comprising contacting the composition with a buffering agent.

271. The method of claim 270, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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272. The method of any one of claims 256-271, further comprising contacting the composition with sterile water.

273. The method of any one of claims 256-272, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

274. The method of any one of claims 256-273, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

275. The method of any one of claims 256-274, wherein the immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

276. The method of any one of claims 256-275, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

277. The method of any one of claims 256-276, wherein the composition further comprises a preservative.

278. The method of any one of claims 256-277, wherein the composition further comprises an anti-fungal compound or a salt thereof.

279. The method of any one of claims 256-278, wherein the composition further comprises a salt.

280. The method claim 279, wherein the salt is an inorganic salt.

281. The method of any one of claims 256-280, wherein the immunogenic saccharide-polypeptide conjugate of the composition is isolated and purified.

282. The method of any one of claims 256-281, wherein the composition is used as a vaccine.

283. The method of any claims 256-282, wherein the composition is used for vaccinating a subject.

284. The method of claim 283, wherein the subject is a human.

285. The method of any one of claims 267-284, wherein the subject or human is a subject or human in need thereof.

286. The method of any one of claims 256-285, wherein the immunogenic saccharide-polypeptide conjugate(s) of the composition are individually, respectively, or collectively

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present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

287. The method of any one of claims 256-286, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using bacteria.

288. The method of any one of claims 256-287, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using E. coli.

289. The method of any one of claims 256-288, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

290. The method of any one of claims 256-289, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

291. The method of any one of claims 256-290, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

292. A kit comprising of a pharmaceutical composition of any of claims 1-111 contained in a container.

293. The kit of claim 292, further comprising instructions for use.

294. The kit of any one of claims 292-293, wherein the container is a syringe.

295. The kit of any one of claims 292-294, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

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

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Published:

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WO 2017/173415 A2

(54) **Title**: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

(57) **Abstract**: Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

Petitioner Merck Ex-1004, 0283

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 503087-01601	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2017/025621	International filing date *(day/month/year)* 31 March 2017 (31.03.2017)	Priority date *(day/month/year)* 31 March 2016 (31.03.2016)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant LIFFEY BIOTECH LIMITED		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 02 October 2018 (02.10.2018)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pct.team6@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Keli L. Holzapfel
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 SEP 2017
Applicant's or agent's file reference 503087-01601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US17/25621	31 March 2017 (31.03.2017)	31 March 2016 (31.03.2016)

International Patent Classification (IPC) or both national classification and IPC
IPC - A61K 39/09, 39/02, 39/385; C07K 14/315 (2017.01)
CPC - A61K 39/092, 39/02, 47/646, 39/385; C07K 14/3156, 16/1271

Applicant LIFFEY BIOTECH LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	22 August 2017 (22.08.2017)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 71-162, 168-295

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 71-162, 168-295 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 71-162, 168-295 are all dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 71-162, 168-295

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-70, 163-167, and S. pneumoniae serotype 2 are directed toward pharmaceutical compositions comprising a plurality of immunogenic saccharide-polypeptide conjugates; and a method comprising administering a second composition at least 4 weeks after a first composition.

The compositions, method of making and administration method will be searched to the extent they encompass a S. pneumoniae serotype encompassing serotype 2 (first exemplary serotype). Applicant is invited to elect additional serotype(s) to be searched. Additional serotype(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 23 (in-part), 24 (in-part), 67 (in-part), 70 (in-part) and 163-167 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass serotype 2 (serotype). Applicants must specify the claims that encompass any additionally elected serotype(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a serotype encompassing serotype 6C (first exemplary elected serotype).

No technical features are shared between the S. penumoniae serotypes of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide; and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

However, these shared technical features are previously disclosed by US 2015/0374811 A1 to Children's Medical Center Corporation (hereinafter 'CMCC').

-***-Continued Within the Next Supplemental Box-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-3, 23-24, 67, 70, 163-167

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	23/1-3, 163-166, 167/163-166	YES
	Claims	1-3, 24, 67, 70	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	NO
Industrial applicability (IA)	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3, 24, 67 and 70 lack novelty under PCT Article 33(2) as being anticipated by US 2015/0374811 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) (hereinafter 'Children').

As per claim 1, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 2, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 3, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 24, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least 28 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 28 unique (pathogenic) immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraphs [0055], [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 67, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising an immunogenic saccharide-polypeptide conjugate (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]) comprising, individually, at least two unique capsular polysaccharides conjugated to a polypeptide (unique immunogenic saccharide-polypeptide conjugates each comprising one or more capsular polysaccharides conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the at least two unique capsular polysaccharides are from a Streptococcus pneumoniae serotype selected from a group consisting of 1 (paragraph [0134]), 2 (paragraph [0134]), 3 (paragraph [0134]), 4 (paragraph [0134]), 5 (paragraph [0134]), 6B (paragraph [0134]), 7F (paragraph [0134]), 9V (paragraph [0134]), 14 (paragraph [0134]), 18C (paragraph [0134]), 19A (paragraph [0134]), 19F (paragraph [0134]), 23F (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

As per claim 70, Children discloses the pharmaceutical composition of claim 67, and Children further discloses comprising at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14 (capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14; paragraph [0134]).

Claims 23/1-3 lack an inventive step under PCT Article 33(3) as being obvious over Children.

As per claims 23/1-3, Children discloses the pharmaceutical composition of claims 1-3, and Children further discloses wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (unique Streptococcus pneumoniae serotype can be 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 22F, 23A, 24F, 33F and 34; paragraph [0134]). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including that unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, based on the previous disclosure of Children that any S. pneumoniae capsular polysaccharide can be used in the immunogenic composition (Children; paragraph [0134]).

Claims 163-164 and 167/163-164 lack an inventive step under PCT Article 33(3) as being obvious over Children in view of the publication entitled 'Experience with Pneumococcal Polysaccharide Conjugate Vaccine (Conjugated to CRM197 Carrier Protein) in Children and Adults' by Durando et al. (hereinafter 'Durando').

As per claim 163, Children discloses a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (secondary immunization with the immunogenic composition after the first immunization; paragraph [0419]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide, where each conjugate can have 75-99 percent homology; paragraphs [0134], [0232]-[0233]); and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype (first immunogenic saccharide-polypeptide conjugate composition can comprise a first serotype picked from any of the listed 28 different serotypes; paragraphs [0055], [0134], [0266]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype (second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype specifically 6B, 14F or 19F; paragraphs [0134], [0266]), and wherein the first serotype and the second serotype are different (first and second serotypes can be different; paragraphs [0134], [0266]). Children does not disclose administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered. Durando discloses administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered (patient receives 2 different doses of immunogenic saccharide-polypeptide conjugates 4 weeks apart; page 7, column 1, paragraph 2). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including the time interval between 2 doses, as disclosed by Durando, for providing the advantage of priming and boosting the immune response using different antigens.

As per claim 164, Children and Durando, in combination, disclose the method of claim 163, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
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-***-Continued from Previous Supplemental Box-***-

As per claims 167/163-164, Children and Durando, in combination, disclose the method of claims 163-164, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 165-166 and 167/165-166 lack an inventive step under PCT Article 33(3) as being obvious over Children and Durando in view of the publication entitled 'Functional Analysis of Glycosyltransferases Encoded by the Capsular Polysaccharide Biosynthesis Locus of Streptococcus pneumoniae Serotype 14' by Kolkman et al. (hereinafter 'Kolkman').

As per claim 165, Children and Durando, in combination, disclose the method of claim 164, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]). Children does not disclose wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide. Kolkman discloses wherein the immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claim 166, Children, Durando and Kolkman, in combination, disclose the method of claim 165. Children does not disclose wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. Kolkman discloses wherein the fragment of the capsular polysaccharide comprises a monosaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claims 167/165-166, Children, Durando and Kolkman, in combination, disclose the method of claims 165-166, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 1-3, 23/1-3, 24, 67, 70, 163-166 and 167/163-166 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Box IV: Lack of Unity of Invention-***-

CMCC discloses a pharmaceutical composition (a pharmaceutical composition; paragraph [0026]) comprising at least 2 immunogenic saccharide-polypeptide conjugates (comprising at least 2 immunogenic saccharide-polypeptide conjugates; paragraphs [0134], [0197]) each comprising individually a capsular polysaccharide conjugated to a polypeptide (each comprising individually a capsular polysaccharide conjugated to a polypeptide; paragraph [0134]); and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered; paragraphs [0134], [0162]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (wherein different capsular polysaccharides are conjugated to a single antigen polypeptide (wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition); paragraph [1034]); and wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation (wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation; paragraph [0197]). CMCC further discloses wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance (wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance; paragraph [0162]).

CMCC does not disclose: at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. However, It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have included a specific period of time between administration of a first dose of an immunogenic composition and a second dose of a related immunogenic composition in order to ensure the production of an immune response therebetween, including a period of from 3 weeks to about 6 weeks, such as 4 weeks. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have provided a booster administration of an immunogenic composition comprising antigens from a different serotype of S. pneumoniae, based on the disclosure of CMCC, in order to ensure the production of effective immune responses to one or more seasonal variants of S. pneumoniae, thereby effectively producing year-round immunity in a subject.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the CMCC reference, unity of invention is lacking.

503087-01601

PCT REQUEST

Original (for **SUBMISSION**)

0	**For receiving Office use only**	
0-1	International Application No.	PCT/US17/25621
0-2	International Filing Date	31 MAR 2017 (31.03.2017)
0-3	Name of receiving Office and "PCT International Application"	RO/US

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT-SAFE [EFS-Web mode] Version 3.51.076.252 MT/FOP 20170101/0.20.5.24
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	**Applicant's or agent's file reference**	503087-01601
I	**Title of Invention**	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
II	**Applicant**	
II-1	This person is	Applicant only
II-2	Applicant for	All designated States
II-4	Name	LIFFEY BIOTECH LIMITED
II-5	Address	c/o Mathesons 70 Sir John Rogersons Quay D02 R296 Dublin 2 Ireland
II-6	State of nationality	IE
II-7	State of residence	IE
II-10	e-mail	patentdocket@wsgr.com
II-10(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	as advance copies followed by paper notifications

503087-01601

PCT REQUEST

Original (for **SUBMISSION**)

III-1	**Applicant and/or inventor**	
III-1-1	This person is	Applicant and inventor
III-1-2	Applicant for	All designated States
III-1-4	Name (LAST, First)	FORREST, Bruce D.
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III-1-7	State of residence	US
III-2	**Applicant and/or inventor**	
III-2-1	This person is	Applicant and inventor
III-2-2	Applicant for	All designated States
III-2-4	Name (LAST, First)	LOVE, Jack D.
III-2-5	Address	4111 W. Inman Avenue Tampa, Florida 33609 United States of America
III-2-6	State of nationality	US
III-2-7	State of residence	US
IV-1	**Agent or common representative; or address for correspondence**	
	The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	Agent
IV-1-1	Name (LAST, First)	HOLZAPFEL, Keli L.
IV-1-2	Address	Wilson Sonsini Goodrich & Rosati 650 Page Mill Road Palo Alto, California 94304 United States of America
IV-1-3	Telephone No.	650-493-9300
IV-1-4	Facsimile No.	650-493-6811
IV-1-5	e-mail	patentdocket@wsgr.com
IV-1-6	Agent's registration No.	65,254
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	MCANDREW, Christopher(65,089); ANDRES, JR., Charles(57,537); BAILEY, F. Pinar(71,605); BAJPAI, Reena(69,996); BAL, Colleen(41,596); HOSTETLER, Michael(47,664); NORVIEL, Vernon(32,483)
V	**DESIGNATIONS**	
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**	

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PCT REQUEST

Original (for **SUBMISSION**)

VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	31 March 2016 (31.03.2016)
VI-1-2	Number	62/316,555
VI-1-3	Country or Member of WTO	US
VI-2	**Priority claim of earlier national application**	
VI-2-1	Filing date	02 May 2016 (02.05.2016)
VI-2-2	Number	62/330,245
VI-2-3	Country or Member of WTO	US
VI-3	**Priority claim of earlier national application**	
VI-3-1	Filing date	08 August 2016 (08.08.2016)
VI-3-2	Number	62/372,263
VI-3-3	Country or Member of WTO	US
VI-4	**Priority claim of earlier national application**	
VI-4-1	Filing date	11 August 2016 (11.08.2016)
VI-4-2	Number	62/373,807
VI-4-3	Country or Member of WTO	US
VI-5	**Priority document request**	
	The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1 VI-2 VI-3 VI-4
VI-6	**Incorporation by reference :**	
	where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.	
VII-1	**International Searching Authority Chosen**	United States Patent and Trademark Office (USPTO) (ISA/US)

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VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	–	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	–	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	–	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	–	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	–	
IX	**Check list**	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	5	✓
IX-2	Description	87	–
IX-3	Claims	29	–
IX-4	Abstract	1	✓
IX-5	Drawings	15	–
IX-7	TOTAL	137	
	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	✓	–
IX-20	**Figure of the drawings which should accompany the abstract**		
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	/Keli L. Holzapfel, Reg. No. 65,254/	
X-1-1	Name (LAST, First)	HOLZAPFEL, Keli L.	
X-1-3	Capacity (if such capacity is not obvious from reading the request)		

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PCT REQUEST

Original (for **SUBMISSION**)

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	31 MAR 2017 (31.03.2017)
10-2	**Drawings:**	
10-2-1	Received X	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/US
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

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(74) Agent: **HOLZAPFEL, Keli L.** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (US).

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

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(54) Title: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

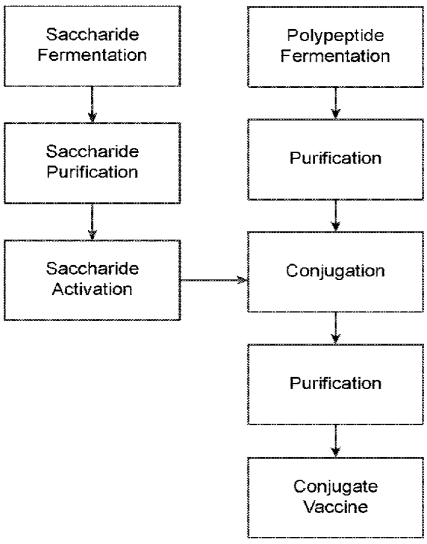


FIG. 1

[Continued on next page]

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TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 14, 2017

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

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Certified by

Michelle K. Lee

Under Secretary of Commerce
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and Director of the United States
Patent and Trademark Office

WSGR Docket No. 50308-701.104

PROVISIONAL PATENT APPLICATION

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

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SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

SUMMARY

[0001] Provided herein is a brief summary. This summary can be used to provide an understanding of embodiments provided herein, but the embodiments are not limited to what is provided in this summary.

[0002] Provided herein is a pharmaceutical composition, which can be in unit dose form, comprising an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to a polypeptide, and wherein the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 23A, 23B, 24F, 24B, 31, 35F, 35B, and 38; or any combination thereof. A pharmaceutical composition, which can be in unit dose form, comprising a plurality of immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least four serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of *Streptococcus pneumoniae*. Further provided is a method comprising administering to a subject a first composition, wherein the first composition is one of any pharmaceutical composition described herein.

[0003] Provided herein is a method comprising administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

[0004] Provided herein is a pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of

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1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0005] Further provided herein is a pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38. Further provided herein is a pharmaceutical composition, which can be in unit dose form, comprising a plurality of at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0006] Provided herein is a pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to a polypeptide, and wherein the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 23A, 23B, 24F, 24B, 31, 35F, 35B, and 38; or any combination thereof. A pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least four serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of *Streptococcus pneumoniae*. Further provided is a method comprising administering to a subject a first composition, wherein the first composition is one of any pharmaceutical composition described herein.

[0007] Provided herein is a method comprising administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate

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composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

[0008] Provided herein is a pharmaceutical composition comprising at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0009] Further provided herein is a pharmaceutical composition comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38. Further provided herein is a pharmaceutical composition comprising at least 16 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0010] Provided herein is a pharmaceutical composition comprising at least 16 immunogenic saccharide-polypeptide conjugates each comprising a capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0011] Further provided herein is a pharmaceutical composition comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38. Further provided herein is a pharmaceutical composition comprising at least 16 immunogenic saccharide-polypeptide conjugates each comprising a capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

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[0012] Further provided herein is a method of making a composition comprising contacting an immunogenic saccharide-polypeptide conjugate of any one of the described pharmaceutical compositions with an excipient, an adjuvant, or any combination thereof.

[0013] Provided herein is a kit comprising of any pharmaceutical composition described herein or any pharmaceutical composition contained in a container described herein.

[0014] Provided herein are methods of making compositions, methods of immunizing with compositions, and methods of vaccinating with compositions.

INCORPORATION BY REFERENCE

[0015] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

DETAILED DESCRIPTION

[0016] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognizes that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0017] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0018] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For

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example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range covering plus or minus up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" can mean within an acceptable error range for the particular value should be assumed.

[0019] A "unit dose" when used in reference to a pharmaceutical composition can refer to units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect. In some instance the unit dose can contain a diluent; *i.e.*, carrier, or vehicle. In some instances the unit dose can be a physically discrete.

[0020] The term "saccharide" can refer to a saccharide, oligosaccharide or polysaccharide. For example, saccharide can mean a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexasaccharide, a heptasaccharide, an octasaccharide, a nonasaccharide, or a decasaccharide. An oligosaccharide can refer to two to ten saccharides. A polysaccharide can refer to more than ten saccharides.

[0021] The term "polypeptide" can refer to a single linear chain of amino acids held together by amide bonds. A polypeptide can refer to at least two amino acids. A polypeptide can be an oligopeptide, wherein the term "oligopeptide" can refer to a single chain of from two to twenty amino acids joined by amide bonds. A polypeptide can be a protein, wherein the term "protein" can refer to a single chain of fifty or more amino acids held together by amide bonds.

[0022] The term an "immune response" can refer to the production of cytokines and/or antibodies with a degree of specificity for an antigen after administering the antigen to a subject. In some instances, the term "immune response" can refer to any change in immune cells or change caused by immune cells after administering the antigen to a subject. An immune response can be T cell independent, T cell dependent, or both.

[0023] The term "homology" can mean percent sequence identity between a particular nucleic acid or amino acid sequence and another nucleic acid or amino acid sequence. First, a nucleic acid or amino acid sequence can be compared another sequence using the BLAST 2 Sequences (Bl2seq) program from the stand- alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. BLASTN can be used to compare nucleic acid sequences, while BLASTP can be used to compare amino acid sequences. Once aligned, the number of matches is

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determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity or homology can be determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (*e.g.*, 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100%.

[0024] The term "length homology" can mean percent length identity between the length of a particular polypeptide and the length of another polypeptide. "Length homology" can in some instances be calculated by dividing the number of amino acids in a first peptide chain by the number of amino acids in a second peptide chain and multiplying the result by 100%. Chain amino acids can be those forming the backbone of the peptide.

[0025] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0026] The term "immunogenic fragment" can be a fragment that is conjugated to a polypeptide, administered to a subject, and which elicits an immune response. In some instances, the fragment can be a fragment of a capsular polysaccharide. In other instances, the polypeptide can be CRM_{197}.

[0027] Immunogenic, which can but need not be obligated to be preceded any term herein, can mean that which, when administered to a subject, which can be a human or an animal, for example a dog, cat, rat, mouse, sheep, or monkey, produces an immune response in the animal. For example, a dog can be *Canis lupis familiarius*, a cat can be *Felis catus*, a rat can be *Rattus norvegicus*, a mouse can be *Mus musculus*, a sheep can be *Ovis aries*, or a monkey can be *Simia inuus*. The administration can be, for example, subcutaneous, intramuscular, or rectal, for example in the form of a suppository.

[0028] In some embodiments, a unique immunogenic saccharide-polypeptide conjugate can be one that differs from other unique immunogenic saccharide-polypeptide conjugates, for example in a plurality of these, by at least one aspect. For example, the unique immunogenic saccharide-polypeptide conjugate can differ by the saccharide or fragment thereof it contains - in that this can render the unique immunogenic saccharide-polypeptide conjugate unique, or by the polypeptide it contains, or both. In some instances, unique immunogenic saccharide-polypeptide conjugates, to be unique, simply need not be 100% identical.

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[0029] The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide.

[0030] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0031] The term "plurality" can mean two or more.

[0032] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

[0033] Bacteria and fungi can incorporate saccharides into their surface structure. For example, *Streptococcus pneumoniae* (*S. pneumoniae*) can contain saccharides in its surface structure. The saccharides can be antigens that can induce an immune response in host, such as an animal or human. For example, B cells can produce antibodies against a saccharide. This response can be a T cell independent immune response. An immune response can be induced with full-length, native form polysaccharides, oligosaccharides, or fragments thereof. An immune response can be induced with full-length, native form capsular polysaccharides, or fragments thereof. Other bacteria that can contain saccharides are *Neisseria meningitidis (N. meningitidis)*, *Salmonella enterica (S. enterica)*, and *Haemophilus influenzae (H. influenzae or "Hib")*.

[0034] The saccharides on a bacteria or fungi can be used to classify the bacteria or fungi into a subclass or serotype. More specifically, a serotype can refer to the type of a microorganism determined by its constituent antigens. The constituent antigens can be different saccharides, such as different capsular polysaccharides or immunogenic fragments thereof. Some non-limiting examples of *S. pneumoniae* serotypes can include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0035] By conjugating a saccharide to a polypeptide, the immune response to the saccharide can be enhanced by inducing a T cell dependent response. A saccharide alone may not stimulate T cells because, for example, a saccharide may not be loaded onto the Major Histocompatibility Complex (MHC) of antigen presenting cells. However, peptides can be loaded onto MHC. Therefore, by conjugating a saccharide to a polypeptide, the saccharide-polypeptide conjugate can then be loaded onto the MHC, recognized by the T cell, and stimulate a T cell response. T cells can then stimulate a more vigorous immune response and also promote a more rapid and long-lasting immunologic memory. Provided herein are saccharide-polypeptide conjugates that

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can be used to induce an immune response, which can be referred to as immunogenic saccharide-polypeptide conjugates. In some instances, the immunogenic saccharide-polypeptide conjugates can provide immune protection against a saccharide or organism containing the saccharides. In other instances, the immunogenic saccharide-polypeptide conjugates can be part of a vaccine.

Compositions of saccharide-polypeptide conjugate

[0036] An immunogenic saccharide-polypeptide conjugate composition can be a saccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide or fragment of the capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise a saccharide antigen coupled to one or more polypeptides. A saccharide antigen can elicit an immune response.

[0037] A saccharide can be from any bacteria and fungi that can incorporate saccharides into their surface structure. For example, saccharides can be from *S. pneumoniae*. Further examples include saccharides from *N. meningitidis*, *S. enterica*, or *H. influenzae*. A saccharide can be a full-length, native form polysaccharide, oligosaccharide, or fragments thereof. A saccharide can be in any form that induces an immune response. A saccharide can be a capsular polysaccharide or fragment thereof. A saccharide can be from a subclass or serotype. Some non-limiting examples of saccharides that are *S. pneumoniae* serotypes include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 18C, 19F, 19A, 23F, 23A, 23B, 15A, 15B, 15C, 16F, 17F, 20, 22F, 24F, 24B, 31, 33F, 35F, 35B, and 38.

[0038] Saccharides can be isolated from bacteria or fungi. Saccharides can be isolated from bacteria or fungi and then conjugated to a polypeptide to form a saccharide-polypeptide conjugate. Any method for isolating the unconjugated polysaccharide can be used. For example, a polysaccharide serotype can be grown in a soy-based medium. The individual polysaccharides can then be purified by steps including centrifugation, precipitation, and ultra-filtration. *See* U.S. Patent Application Publication No. 2008/028638 and U.S. Pat. No. 5,847,112. Other processes for purifying polysaccharides are described in U.S. Pat. No. 4,686,102; 4,242,501; 4221,906; 5623,057; and 5,847,112. Alternatively or additionally, polysaccharides may be isolated simultaneously from more than one serotype, which can produce a mixture of polysaccharide serotypes with the desired polysaccharide already combined. Furthermore, these isolated

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polysaccharides can be sized by microfluidization. The polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated sample. A saccharide-polypeptide conjugate can include a saccharide or a fragment thereof which is at least partially embedded in one or more polypeptides. The one or more polypeptides can have one or more crosslinks. The at least partially embedded saccharide or fragment thereof can, but need not be, covalently bound to at least one of the polypeptides when at least partially embedded therein.

[0039] Saccharides that can be used in the saccharide-polypeptide conjugate can also be obtained from commercial sources. For example, individual polysaccharide serotypes can be obtained in a powder form. These polysaccharides can be dissolved in water and incubated with a salt to dissociate residual impurities, which can then be removed by a filtration step. The purified polysaccharides serotypes can then be conjugated to a polypeptide.

[0040] A polypeptide of an immunogenic saccharide-polypeptide conjugate can be an oligopeptide. A polypeptide of can be a carrier protein. A polypeptide can be any polypeptide that allows for conjugation or coupling of saccharide and results in the display of the conjugate or coupling in a way that induces a protective immune response against the saccharide. A polypeptide can be any polypeptide that allows for conjugation or coupling of a capsular polysaccharide or fragment of the capsular polysaccharide and can result in the display of the conjugate or coupling in a way that can induce a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide. A polypeptide can be CRM_{197}, tetanus toxoid, a diphtheria toxoid, a cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, a keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), and protein D from *H. influenza*, or any fragment thereof. The polypeptide can be a polypeptide in which the toxin activity of the polypeptide can be at least partially mitigated. Examples of suitable polypeptides for use as in a saccharide-polypeptide conjugate are described in U.S. patent 8,808,707 and can include: polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, *e.g.*, serines, threonines, or tyrosines, to confer desirable solubility properties on the resultant

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loaded carrier and conjugate. Additionally, suitable polypeptide carriers can include bacterial toxins, toxoids, or inactivated toxin. A toxoid can be a bacterial toxin whose toxicity has been weakened or suppressed while other properties, typically immunogenicity, are maintained. As a class, bacterial toxins and derivatives thereof can to be highly immunogenic. Polypeptides derived from bacterial toxins can be effective at eliciting an immune response to saccharides of the conjugate. Steps can be taken (*e.g.*, by chemical and/or genetic means) to render the toxins non-toxic and safe for administration to mammals. Examples of such bacterial toxin-derived polypeptides which may currently commonly used in vaccine compositions, and can be used in the immunogenic saccharide-polypeptide conjugate described herein, include the diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof. Polypeptide fragments can be used to elicit a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide of the immunogenic saccharide-polypeptide conjugate. These fragments can encompass T-helper epitopes.

[0041] The saccharides can be conjugated to the polypeptide by, *e.g.*, chemical or biological synthesis. A capsular polysaccharide or fragment of the capsular polysaccharide can be conjugated to the polypeptide by chemical or biological synthesis.

[0042] For example, the capsular polysaccharide or immunogenic fragment thereof can be chemically activated and then reacted with the polypeptide. For example, capsular polysaccharides or fragments of the capsular polysaccharides can be reacted with either ammonia or diaminoethane to generate a free terminal amino group. The amino group can then be reacted with an excess of disuccininidy ester of adipic acid, which can introduce an active succininmidyl ester group. This can then be reacted to a free amino group on the polypeptide to form covalent amide bonds. As another example, the immunogenic saccharide-polypeptide conjugate can be produced using bacteria, such as *Escherichia coli* (*E. coli*). The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially embedded in the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide

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chemically cross-linked to the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially chemically cross-linked to the polypeptide. A capsular polysaccharide or fragment of the capsular polysaccharide of a serotype from *S. pneumoniae* can be conjugated to a polypeptide by chemical or biological synthesis. One saccharide can be conjugated to one polypeptide. Two or more saccharides can be conjugated to one polypeptide. The saccharides can be the same serotype. The saccharides can be different serotypes. One capsular polysaccharide or immunogenic fragment thereof can be conjugated to one polypeptide. Two or more capsular polysaccharides or fragments thereof can be conjugated to one polypeptide. The capsular polysaccharides or fragments thereof can be the same serotype. The capsular polysaccharides or fragments thereof can be different serotypes.

[0043] A polypeptide can be conjugated to an immunogenic serotype 1 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 2 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 3 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 4 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 5 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 6C capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 7F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 8 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 9N capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 9V capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 10A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae.* A polypeptide can be conjugated to an immunogenic serotype 11A capsular polysaccharide or fragment thereof

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isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 12F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 14 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15C capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 16F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 17F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 18C capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 19A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 19F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 20 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 20F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 22F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 24F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 24B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 31 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 33F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 35F

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capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 35B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 38 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*.

[0044] In some embodiments, CRM_{197} can be conjugated to an immunogenic serotype 1 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 2 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 3 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 4 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 5 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6C capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 7F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 8 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9N capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9V capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 10A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 11A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 12F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 14 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15C capsular

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polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 16F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 17F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 18C capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 22F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 31 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 33F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35F capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35B capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 38 capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*.

[0045] In further embodiments, any of the saccharides as described herein can be conjugated to any polypeptides described herein. Any of the immunogenic saccharide-polypeptide conjugates described herein can be isolated and purified.

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Pharmaceutical compositions

[0046] The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition. The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition in unit dose form. The saccharide-polypeptide conjugates described herein can be in the form of a vaccine. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can be in unit dose form and can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate wherein the capsular polysaccharide or immunogenic fragment thereof of the saccharide-polypeptide conjugate can be one serotype. In another embodiment, a pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the capsular polysaccharides or immunogenic fragments thereof can be from different serotypes, creating a mixture of different immunogenic saccharide-polypeptide conjugates. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugates wherein the polypeptide is the same polypeptide in all the immunogenic saccharide-polypeptide conjugates. In other embodiments, a pharmaceutical composition can comprise immunogenic saccharide-polypeptide conjugates wherein the polypeptide(s) in the saccharide-polypeptide conjugates are a mixture of different polypeptide(s).

[0047] For example, a pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 35F, 35B, and 38; or a combination thereof. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, , 22F 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38; or a combination thereof. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes selected from the group consisting of 2,

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6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38. A pharmaceutical composition can comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the

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serotypes 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B 31, 33F, 35B, 35F, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B 31, 33F, 35B, 35F, and 38. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 20, 22F, 23A, 23B, 24B, 31, 33F, 35B, 35F, and 38. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 33F, 35F, 35B, or 38. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B,

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23F, 24F, 33F, 35F, 35B, or 38. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, 35B. The immunogenic saccharide-polypeptide conjugate(s) can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include excipients that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The immunogenic saccharide-polypeptide conjugate(s) can include adjuvants that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition. The pharmaceutical composition can be administered to one of a plurality of humans, which elicits at least one of the following side effects in less than about 5%, 10%, 20% or 30% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

[0048] In other embodiments, a pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide is from *N. meningitidis*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *S. enterica*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *H. influenzae*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *Neisseria meningitidis*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *S. enterica*. A pharmaceutical composition can comprise

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can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *H. influenzae*.

[0049] The polypeptide of any of the plurality of saccharide-polypeptide conjugates can be the same polypeptide. For example, the polypeptide of any of the plurality of saccharide-polypeptide conjugates can be CRM_{197}. The plurality of saccharide-polypeptide conjugates can comprise a mixture of different polypeptides. For example, the plurality of saccharide-polypeptide conjugates can comprise a mixture of CRM_{197}, tetanus toxoid, a diphtheria toxoid, or any fragment thereof.

[0050] Any pharmaceutical composition described herein can be in unit dose form. Additionally, the pharmaceutical composition can further comprise an adjuvant. In some embodiments, the adjuvant can be an aluminum-cation comprises adjuvant. In other embodiments, the adjuvant can be aluminum phosphate, aluminum sulfate, or aluminum hydroxide. More examples of suitable adjuvants for use in the pharmaceutical composition include: aluminum-based salts and variants thereof; emulsions (either water-in-oil or oil-in-water) and variants thereof, *e.g.*, Freund's Incomplete Adjuvant, MF59® (Glaxosmithkline plc); PRR ligands and variants thereof, *e.g.*, pathogen associated molecular patterns (PAMPs); TLR3 and RLR ligands or variants thereof, *e.g.*, Synthetic analogs of dsRNA, poly(I:C), etc.; TLR4 ligands or variants thereof, *e.g.*, bacterial flagellin, Glucopyranosyl Lipid Adjuvant (GLA), monophosphoryl lipid A, etc.; TLR5 ligands or variants thereof, *e.g.*, imiquimod, gardiquimod, R848, etc.; TLR9 ligands or variants thereof, *e.g.*, Oxydeoxynucleotides containing CpG motifs (CpG ODNs such as ODN1826 and ODN2006); NOD2 ligands or variants thereof, *e.g.*, fragments of bacterial cell walls (such as muramyl dipeptide [MDP]); saponins including synthetic derivatives or variants thereof, e.g., QS21, TQL1055, etc.); or any combination of above.

[0051] A pharmaceutical composition can further comprise an excipient. An excipient can be Acacia, Acesulfame Potassium, Acetic Acid, Glacial Acetone, Acetyltributyl Citrate, Acetyltriethyl Citrate, Agar, Albumin, Alcohol, Alginic Acid, Aliphatic Polyesters, Alitame, Almond Oil, Alpha Tocopherol, Aluminum Hydroxide Adjuvant, Aluminum Oxide, Aluminum Phosphate Adjuvant, Aluminum Stearate, Ammonia Solution, Ammonium Alginate, Ascorbic Acid, Ascorbyl Palmitate, Aspartame, Attapulgite, Bentonite, Benzalkonium Chloride, Benzethonium Chloride, Benzoic Acid, Benzyl Alcohol, Benzyl Benzoate, Boric Acid, Bronopol, Butylated Hydroxyanisole, Butylated Hydroxytoluene, Butylparaben, Calcium

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Alginate, Calcium Carbonate, Dibasic Anhydrous Calcium Phosphate, Dibasic Dihydrate Calcium Phosphate, Tribasic Calcium Phosphate, Calcium Stearate, Calcium Sulfate, Canola Oil, Carbomer, Carbon Dioxide, Carboxymethylcellulose Calcium, Carboxymethylcellulose Sodium, Carrageenan, Castor Oil, Hydrogenated Castor Oil, Microcrystalline Cellulose, Powdered Cellulose, Silicified Microcrystalline Cellulose, Cellulose Acetate, Cellulose Acetate Phthalate, Ceratonia, Cetostearyl Alcohol, Cetrimide, Cetyl Alcohol, Cetylpyridinium Chloride, Chitosan, Chlorhexidine, Chlorobutanol, Chlorocresol, Chlorodifluoroethane (HCFC), Chlorofluorocarbons (CFC), Chloroxylenol, Cholesterol, Citric Acid Monohydrate, Colloidal Silicon Dioxide, Coloring Agents, Copovidone, Corn Oil, Cottonseed Oil, Cresol, Croscarmellose Sodium, Crospovidone, Cyclodextrins, Cyclomethicone, Denatonium Benzoate, Dextrates, Dextrin, Dextrose, Dibutyl Phthalate, Dibutyl Sebacate, Diethanolamine, Diethyl Phthalate, Difluoroethane (HFC), Dimethicone, Dimethyl Ether, Dimethyl Phthalate, Dimethyl Sulfoxide, Dimethylacetamide, Disodium Edetate, Docusate Sodium, Edetic Acid, Erythorbic Acid, Erythritol, Ethyl Acetate, Ethyl Lactate, Ethyl Maltol, Ethyl Oleate, Ethyl Vanillin, Ethylcellulose, Ethylene Glycol Palmitostearate, Ethylene Vinyl Acetate, Ethylparaben, Fructose, Fumaric Acid, Gelatin, Glucose, Liquid, Glycerin, Glyceryl Behenate, Glyceryl Monooleate, Glyceryl Monostearate, Glyceryl Palmitostearate, Glycofurol, Guar Gum, Hectorite, Heptafluoropropane (HFC), Hexetidine, Hydrocarbons (HC), Hydrochloric Acid, Hydroxyethyl Cellulose, Hydroxyethylmethyl Cellulose, Hydroxypropyl Cellulose, Low-substituted Hydroxypropyl Cellulose, Hydroxypropyl Starch, Hypromellose, Hypromellose Acetate Succinate, Hypromellose Phthalate, Imidurea, Inulin, Iron Oxides, Isomalt, Isopropyl Alcohol, Isopropyl Myristate, Isopropyl P,almitate, Kaolin, Lactic Acid, Lactitol, Anhydrous Lactose, Monohydrate Lactose, Spray-Dried Lactose, Lanolin, Lanolin Alcohols, Hydrous Lanolin, Lauric Acid, Lecithin, Leucine, Linoleic Acid, Macrogol, Hydroxystearate Magnesium Aluminum Silicate, Magnesium Carbonate, Magnesium Oxide, Magnesium Silicate, Magnesium Stearate, Magnesium Trisilicate, Malic Acid, Maltitol, Maltitol Solution, Maltodextrin, Maltol, Maltose, Mannitol, Medium-chain Triglycerides, Meglumine, Menthol, Methylcellulose, Methylparaben, Mineral Oil, Light Mineral Oil, Mineral Oil and Lanolin Alcohols, Monoethanolamine, Monosodium Glutamate, Monothioglycerol, Myristic Acid, Neohesperidin Dihydrochalcone, Nitrogen, Nitrous Oxide, Octyldodecanol, Oleic Acid, Oleyl Alcohol, Olive Oil, Palmitic Acid, Paraffin, Peanut Oil, Pectin, Petrolatum, Petrolatum and Lanolin Alcohols,

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Phenol, Phenoxyethanol, Phenylethyl Alcohol, Phenylmercuric Acetate, Phenylmercuric Borate, Phenylmercuric Nitrate, Phosphoric Acid, Polacrilin Potassium, Poloxamer, Polycarbophil, Polydextrose, Polyethylene Glycol, Polyethylene Oxide, Polymethacrylates, Poly(methyl vinyl ether/maleic anhydride), Polyoxyethylene Alkyl Ethers, Polyoxyethylene Castor Oil Derivatives, Polyoxyethylene Sorbitan Fatty Acid Esters, Polyoxyethylene Stearates, Polyvinyl Acetate Phthalate, Polyvinyl Alcohol, Potassium Alginate, Potassium Benzoate, Potassium Bicarbonate, Potassium Chloride, Potassium Citrate, Potassium Hydroxide, Potassium Metabisulfite, Potassium Sorbate, Povidone, Propionic Acid, Propyl Gallate, Propylene Carbonate, Propylene Glycol, Propylene Glycol Alginate, Propylparaben, 2-Pyrrolidone, Raffinose, Saccharin, Saccharin Sodium, Saponite, Sesame Oil, Shellac, Simethicone, Sodium Acetate, Sodium Alginate, Sodium Ascorbate, Sodium Benzoate, Sodium Bicarbonate, Sodium Borate, Sodium Chloride, Sodium Citrate Dihydrate, Sodium Cyclamate, Sodium Hyaluronate, Sodium Hydroxide, Sodium Lactate, Sodium Lauryl Sulfate, Sodium Metabisulfite, Dibasic Sodium Phosphate, Monobasic Sodium Phosphate, Sodium Propionate, Sodium Starch Glycolate, Sodium Stearyl Fumarate, Sodium Sulfite, Sorbic Acid, Sorbitan Esters (Sorbitan Fatty Acid Esters), Sorbitol, Soybean Oil, Starch, Pregelatinized Starch, Sterilizable Maize Starch, Stearic Acid, Stearyl Alcohol, Sucralose, Sucrose, Compressible Sugar, Confectioner's Sugar, Sugar Spheres, Sulfobutylether b-Cyclodextrin, Sulfuric Acid, Sunflower Oil, Hard Fat Suppository Bases, Talc, Tartaric Acid, Tetrafluoroethane (HFC), Thaumatin, Thimerosal, Thymol, Titanium Dioxide, Tragacanth, Trehalose, Triacetin, Tributyl Citrate, Triethanolamine, Triethyl Citrate, Vanillin, Hydrogenated Vegetable Oil, Water, Anionic Emulsifying Wax, Carnauba Wax, Cetyl Esters Wax, Microcrystalline Wax, Nonionic Emulsifying Wax, White Wax, Yellow Wax, Xanthan Gum, Xylitol, Zein, Zinc Acetate, or Zinc Stearate.

[0052] A pharmaceutical composition can further comprise a surfactant or emulsifier, or a combination thereof. A surfactant can be polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. In some embodiments, polysorbate can be polysorbate 80. In other embodiments, polymer glycol can be polyethylene glycol. A pharmaceutical composition can comprise a specific polyethylene glycol based upon its molecular weight. A pharmaceutical composition can comprise a preservative. A pharmaceutical composition can comprise a salt. The salt is an inorganic salt. A pharmaceutical composition can further comprise an anti-fungal compound or a salt thereof. An antifungal compound can be thimerosal, phenol, benzethonium

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chloride, or 2-phenoxyethanol. A pharmaceutical composition can further comprise a chelating agent. A chelating agent can be ethylenediainetetraacetic acid (EDTA). A pharmaceutical composition can further comprise a buffering agent. The buffering agent is succinate buffer agent, tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, phosphate buffering agent, sodium phosphate buffering agent, or a potassium phosphate buffering agent. A pharmaceutical composition can further comprise sterile water. When the pharmaceutical composition can be administered intramuscularly to a human, the pharmaceutical composition can elicit an immune response that lasts from about 1 week to about 30 years. A pharmaceutical composition retains at least about 60% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 70% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition can retain at least about 90% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

Methods of Use

[0053] The saccharide-polypeptide conjugates as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be used in a vaccine. The vaccine can be administered to a subject. The subject can be a human. The subject or human can be a subject or human in need thereof.

[0054] The saccharide-polypeptide conjugate can be administered by intramuscular injection. The saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal,

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intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The saccharide-polypeptide conjugate can be administered via a suppository. The saccharide-polypeptide conjugate can be administered intranasally.

[0055] The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered by intramuscular injection. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The pharmaceutical composition comprising a saccharide-polypeptide conjugate can be administered intranasally. The pharmaceutical composition can be administered intramuscularly and can elicit an immune response that lasts from 1 week to 30 years.

[0056] For example, a pharmaceutical composition administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, , 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38; or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38. A pharmaceutical composition administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24B, 24F, 31, 33F, 35B, 35F, and 38. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can

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comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38, or a combination thereof. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38, or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, or a combination thereof. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 additional serotype

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comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35B, or 38. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 35F, 35B, or 38. A pharmaceutical composition can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, 35B.

[0057] Two different pharmaceutical compositions can be administered to a subject. One pharmaceutical composition can be administered to the subject at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years before or at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years after an administration to the subject a second pharmaceutical composition. In some embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at less than 70% length homology, 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

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[0058] In some embodiments, the first pharmaceutical composition can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38; or a combination thereof. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24B, 24F, 31, 33F, 35B, 35F, and 38. The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N,

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10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

[0059] In some embodiments, the second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24B, 24F, 31, 33F, 35B, 35F, and 38, wherein the serotype of the first pharmaceutical composition and the serotype of the second pharmaceutical composition are distinct.

[0060] In other embodiments, the first pharmaceutical composition and the second pharmaceutical composition can be administered at least about 4 weeks apart, at least 6 about weeks apart, at least 2 about months apart, at a least about 3 months apart, at least about 4 months apart, at least about 5 months apart, at least 6 months apart, at least about 7 months apart, at least about 8 months apart, at least about 9 months apart, at least about 10 months apart, at least 11 about months apart, at least about 1 year apart, at least about 1.5 years apart, at least about 2 years apart, at least about 5 years apart, at least about 10 years apart, at least about 15 year, at least about 20 years apart, or at least about 30 years apart.

[0061] Different serotypes of *S. pneumoniae* can be more pervasive in different geographical areas. For example, a certain group of serotypes can commonly cause pneumococcal disease in the United States and Canada. This group of serotypes can be different for Europe, the Middle East, North Africa, Japan and Korea. For example, administration of a pharmaceutical composition a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 33F, 35F, 35B, and 38 may improve protection against *S. pneumoniae* disease the subject in the United States and/or Canada. Administration of a pharmaceutical composition a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38 may improve protection against *S. pneumoniae* disease the

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subject in the Europe, the Middle East, and/or North Africa. Administration of a pharmaceutical composition a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 15C, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38 may improve protection against *S. pneumoniae* disease the subject in the Japan and/or Korea.

[0062] A method of administration can comprise administering a first saccharide-polypeptide conjugate composition to a subject; administering a second saccharide-polypeptide conjugate composition to the subject at least four weeks after administering the first saccharide-polypeptide conjugate composition; wherein the first saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a second polypeptide of the second saccharide-polypeptide conjugate composition; and wherein the first saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. In some embodiments, the first serotype is from the same bacteria as the second serotype. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition comprises a saccharide-polypeptide conjugate comprising a polypeptide that has at less than about 70% length homology, about 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0063] In other embodiments, the first serotype can be from a different bacteria than the second serotype. The first serotype can be from *Streptococcus pneumoniae*. The first serotype can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. The second serotype can be from *Streptococcus pneumoniae*. The second bacteria can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. For example, the first serotype can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F and 38 from *Streptococcus pneumoniae*. The first serotype can comprise at least one of 2, 6C, 8, 9N,

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10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 from *Streptococcus pneumoniae*, and the second serotype can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

[0064] In other embodiments, the conducting of any of the methods described herein may not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

Saccharide-polypeptide Conjugate Kits

[0065] The saccharide-polypeptide conjugates and pharmaceutical compositions described herein can be contained in a kit. The kit can further comprise instructions for use. The saccharide-polypeptide conjugates and/or pharmaceutical compositions can be stored in a container. The container can be a syringe. The container can be a vial. The container can be a vial with a rubber septum or a septum made of flexible, reclose-able material.

[0066] Also provided herein are the following embodiments. A first embodiment of pharmaceutical composition in unit dose form comprising an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to a polypeptide, and wherein the serotype is at least four of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 35B, 35F and 38. A second embodiment of the pharmaceutical composition of embodiment 1, wherein the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide. A third embodiment of the pharmaceutical composition of embodiment 1, wherein the immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide. A fourth embodiment of the pharmaceutical composition of embodiment 3, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. A fifth embodiment of the pharmaceutical composition of any one of embodiments 1-4, wherein the polypeptide is an oligopeptide. A sixth embodiment of the pharmaceutical composition of any one of embodiments 1-5, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion

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protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof. A seventh embodiment of the pharmaceutical composition of any one of embodiments 1-6, wherein a toxin activity of the polypeptide is at least partly mitigated. An eight embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38. A ninth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38. A tenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. An eleventh embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B. A twelfth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A thirteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A fourteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A,

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23B, 33F, and 35B. A fifteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A sixteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A seventeenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. An eighteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A nineteenth embodiment of the pharmaceutical composition of any one of embodiments 1-7, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A twentieth embodiment of the pharmaceutical composition of any one of embodiments 1-19, further comprising an adjuvant. A twenty-first embodiment of the pharmaceutical composition of embodiment 20, wherein the adjuvant is an aluminum-cation comprising adjuvant. A twenty-second embodiment of the pharmaceutical composition of embodiment 21, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A twenty-third embodiment of the pharmaceutical composition of any one of embodiments 1-22, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition. A twenty-fourth embodiment of the pharmaceutical composition of any one of embodiments 1-23, further comprising a chelating agent. A twenty-fifth embodiment of the pharmaceutical composition of embodiment 24, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA). A twenty-sixth embodiment of the pharmaceutical composition of any one of embodiments 1-25, which is in a form of a suspension. A twenty-

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seventh embodiment of the pharmaceutical composition of any one of embodiments 1-26, which is in the form of a homogenous mixture or a heterogeneous mixture. A twenty-eighth embodiment of the pharmaceutical composition of embodiment 27, wherein the pharmaceutical composition is in a form of a homogenous mixture. A twenty-ninth embodiment of the pharmaceutical composition of any one of embodiments 1-28, which is contained in a pre-filled syringe. A thirtieth embodiment of the pharmaceutical composition of any one of embodiments 1-29, further comprising a surfactant , an emulsifier, or a combination thereof. A thirty-first embodiment of the pharmaceutical composition of embodiment 30, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof. A thirty-second embodiment of the pharmaceutical composition of embodiment 31, comprising the polysorbate, wherein the polysorbate is polysorbate 80.A thirty-third embodiment of the pharmaceutical composition of embodiment 31, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol. A thirty-fourth embodiment of the pharmaceutical composition of any one of embodiments 1-33, further comprising a buffering agent. A thirty-fifth embodiment of the pharmaceutical composition of embodiment 34, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A thirty-sixth embodiment of the pharmaceutical composition of any one of embodiments 1-35, further comprising sterile water. A thirty-seventh embodiment of the pharmaceutical composition of any one of embodiments 1-36, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A thirty-eighth embodiment of the pharmaceutical composition of any one of embodiments 1-37, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years. A thirty-ninth embodiment of the pharmaceutical composition of any one of embodiments 1-38, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

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A fortieth embodiment of the pharmaceutical composition of any one of embodiments 1-19, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

[0067] A forty-first embodiment of a method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of embodiments 1-40. A forty-second embodiment of the method of embodiment 41, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition. A forty-third embodiment of the method of embodiment 41-42, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide. A forty-fourth embodiment of the method of any one of embodiments 41-43, wherein the second composition comprises a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A forty-fifth embodiment of the method of any one of embodiments 41-43, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F. A forty-sixth embodiment of the method of any one embodiments 41-43, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct. A forty-seventh embodiment of the method of embodiment 43, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide. A forty-eighth embodiment of the method of embodiment 43, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises the fragment of the capsular polysaccharide. A forty-ninth embodiment of the method of embodiment 48, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a

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trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. A fiftieth embodiment of the method of any one of embodiments 41-49, wherein the second polypeptide is an oligopeptide. A fifty-first embodiment of the method any one of embodiments 41-48, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof. A fifty-second embodiment of the method of any one of embodiments 41-51, wherein a toxin activity of the second polypeptide is at least partly mitigated. A fifty-third embodiment of the method of any one of embodiments 41-52, wherein the second composition further comprises an adjuvant. A fifty-fourth embodiment of the method of embodiment 53, wherein the adjuvant is an aluminum-cation comprising adjuvant. A fifty-fifth embodiment of the method of claim 53, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A fifty-sixth embodiment of the method any one of embodiments 41-55, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition. A fifty-seventh embodiment of the method of any one of embodiments 41-56, wherein the second composition further comprises a chelating agent. A fifty-eighth embodiment of the method of embodiment 57, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA). A fifty-ninth embodiment of the method any one of embodiments 41-58, wherein the second composition is in a form of a suspension. A sixtieth embodiment of the method any one of embodiments 41-59, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture. A sixty-first embodiment of the method any one of embodiments 41-60, wherein the second composition is in the form of a homogenous mixture. A sixty-second embodiment of the method any one of embodiments 41-61, wherein the second composition is contained in a pre-filled syringe. A sixty-third embodiment of the method any one of embodiments 41-62, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof. A sixty-fourth embodiment of the method embodiment 63,wherein the second composition further

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comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. A sixty-fifth embodiment of the method of embodiment 64, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80. A sixty-sixth embodiment of the method of embodiment 64, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol. A sixty-seventh embodiment of the method any one of embodiments 41-66, wherein the second composition further comprises a buffering agent. A sixty-eighth embodiment of the method of embodiment 67, wherein the buffering agent is a succinate buffer agent, a tris-acetate-ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A sixty-ninth embodiment of the method any one of embodiments 41-68, wherein the second composition further comprises sterile water. A seventieth embodiment of the method any one of embodiments 41-69, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A seventy-first embodiment of the method any one of embodiments 41-70, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years. A seventy-second embodiment of the method of any one of embodiments 41-72, wherein the second composition is administered intramuscularly the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years. A seventy-third embodiment of the method of any one of embodiments 41-73, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0068] A seventy-fourth embodiment of a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence

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homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. A seventy-fifth embodiment of the method of embodiment 74, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide. A seventy-sixth embodiment of the method of embodiment 75, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide. A seventy-seventh embodiment of the method of embodiment 76, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. A seventy-eighth embodiment of the method of any one of embodiments 74-77, wherein the first polypeptide is an oligopeptide. A seventy-ninth embodiment of the method of any one of embodiments 74-78, wherein the second polypeptide is an oligopeptide. An eightieth embodiment of the method of any one of embodiments 74-79, wherein the first polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof . An eighty-first embodiment of the method of any one of embodiments 74-80, wherein a toxin activity of the first polypeptide is at least partly mitigated. An eighty-second embodiment of the method of any one of embodiments 74-81, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenza*, any fragment thereof, or any combination thereof. An eighty-third embodiment of the method of any

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one of embodiments 74-82, wherein a toxin activity of the second polypeptide is at least partly mitigated. An eighty-fourth embodiment of the method of any one of embodiments 74-83, wherein the first serotype is from a same bacteria as the second serotype. An eighty-fifth embodiment of the method of any one of embodiments 74-84, wherein the first serotype is from a different bacteria than the second serotype. An eighty-sixth embodiment of the method of any one of embodiments 74-85, wherein the first serotype is from *Streptococcus pneumoniae*. An eighty-seventh embodiment of the method of any one of embodiments 74-86, wherein the first serotype is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. An eighty-eighth embodiment of the method of any one of embodiments 74-87, wherein the second serotype is from *Streptococcus pneumoniae*. An eighty-ninth embodiment of the method of any one of embodiments 74-88, wherein the second bacteria is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. A ninetieth embodiment of the method of any one of embodiments 74-89, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F,24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*. A ninety-first embodiment of the method of any one of embodiments 74-89, wherein the first serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. A ninety-second embodiment of the method of any one of embodiments 74-91, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant. A ninety-third embodiment of the method of embodiment 92, wherein the adjuvant is an aluminum-cation comprising adjuvant. A ninety-fourth embodiment of the method of embodiment 93, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A ninety-fifth embodiment of the method of any one of embodiments 74-94, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition. A ninety-sixth embodiment of the method of any one of embodiments 74-95, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises

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a chelating agent. A ninety-seventh embodiment of the method of embodiment 96, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA). A ninety-eighth embodiment of the method of any one of embodiments 74-97, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension. A ninety-ninth embodiment of the method of any one of embodiments 74-98, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture. A one hundredth embodiment of the method of any one of embodiments 74-99, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture. A one hundred and first embodiment of the method of any one of embodiments 74-100, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe. A one hundred and second embodiment of the method of any one of embodiments 74-101, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof. A one hundred and third embodiment of the method of embodiment 102, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof. A one hundred and fourth embodiment of the method of embodiment 103, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80. A one hundred and fifth embodiment of the method embodiment 103, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol. A one hundred and sixth embodiment of the method of any one of embodiments 74-106, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent. A one hundred and seventh embodiment of the method of embodiment 106, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A one hundred and eighth embodiment of the method of any one of embodiments 74-107, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water. A one hundred and ninth embodiment of the method of any one of embodiments 74-108, wherein when the first immunogenic saccharide-polypeptide

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conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A one hundred and tenth embodiment of the method of any one of embodiments 74-109, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years. A one hundred and eleventh embodiment of the method of any one of embodiments 74-110, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A one hundred and twelfth embodiment of the method of any one of embodiments 74-111, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant. A one hundred and thirteenth embodiment of the method of embodiment 112, wherein the adjuvant is an aluminum-cation comprising adjuvant. A one hundred and fourteenth embodiment of the method of embodiment 112, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A one hundred and fifteenth embodiment of the method of any one of embodiments 74-114, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition. A one hundred and sixteenth embodiment of the method of any one of embodiments 74-115, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent. A one hundred and seventeenth embodiment of the method of embodiment 116, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA). A one hundred and eighteenth embodiment of the method of any one of embodiments 74-118, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension. A one hundred and nineteenth embodiment of the method of any one of embodiments 74-119, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture. A one hundred and twentieth embodiment of the method of embodiment 119, wherein the second immunogenic saccharide-polypeptide

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conjugate composition is in a form of a homogenous mixture. A one hundred and twenty-first embodiment of the method of any one of embodiments 74-120, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe. A one hundred and twenty-second embodiment of the method of any one of embodiments 74-121, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof. A one hundred and twenty-third embodiment of the method of embodiment 122, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. A one hundred and twenty-fourth embodiment of the method of embodiment 123, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80. A one hundred and twenty-fifth embodiment of the method of embodiment 123, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol. A one hundred and twenty-sixth embodiment of the method of any one of embodiments 74-125, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent. A one hundred and twenty-seventh embodiment of the method of embodiment 126, wherein the buffering agent is a succinate buffer agent, a tris-acetate-ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A one hundred and twenty-eighth embodiment of the method of any one of embodiments 74-127, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water. A one hundred and twenty-ninth embodiment of the method of any one of embodiments 74-128, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A one hundred and thirtieth embodiment of the method of any one of embodiments 74-129, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an

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immune response that lasts from about 1 week to about 30 years. A one hundred and thirty-first embodiment of the method of any one of embodiments 74-130, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A one hundred and thirty-second embodiment of a method of making a composition comprising: contacting the immunogenic saccharide-polypeptide conjugate of any one of embodiments 1-19 with an excipient, an adjuvant, or any combination thereof. A one hundred and thirty-third embodiment of the method of embodiment 132, wherein the composition is injectable or intranasally administrable. A one hundred and thirty-fourth embodiment of the method embodiment 132, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A one hundred and thirty-fifth embodiment of the method of any one of embodiments 132-134, wherein the composition is in the form of an intramuscularly injectable composition. A one hundred and thirty-sixth embodiment of the method of any one of embodiments 132-135, further comprising contacting the composition with a chelating agent. A one hundred and thirty-seventh embodiment of the method of embodiment 136, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA). A one hundred and thirty-eight embodiment of the method of any one of embodiments 132-137, wherein the composition is in a form of a suspension. A one hundred and thirty-ninth embodiment of the method of any one of embodiments 132-138, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture. A one hundred and fortieth embodiment of the method of any one of embodiments 139, wherein the composition is in a form of a homogenous mixture. A one hundred and forty-first embodiment of the method of any one of embodiments 132-140, wherein the composition is contained in a pre-filled syringe. A one hundred and forty-second embodiment of the method of any one of embodiments 132-141, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof. A one hundred and forty-third embodiment of the method of embodiment 142, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof. A one hundred and forty-fourth embodiment of the method of embodiment 143, comprising contacting the

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composition with the polysorbate, wherein the polysorbate is polysorbate 80. A one hundred and forty-fifth embodiment of the method of embodiment 143, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol. A one hundred and forty-sixth embodiment of the method of any one of embodiments 132-145, further comprising contacting the composition with a buffering agent. A one hundred and forty-seventh embodiment of the method of embodiment 146, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A one hundred and forty-eighth embodiment of the method of any one of embodiments 132-147, further comprising contacting the composition with sterile water. A one hundred and forty-ninth embodiment of the method of any one of embodiments 132-148, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A one hundred and fiftieth embodiment of the method of any one of embodiments 132-149, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years. A one hundred and fifty-first embodiment of the method of any one of embodiments 132-150, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0069] A one hundred and fifty-second embodiment of a pharmaceutical composition in unit dose form comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38. A one hundred and fifty-third embodiment of the pharmaceutical composition of embodiment 152, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a capsular polysaccharide. A one hundred and fifty-fourth embodiment of the pharmaceutical composition of embodiment 152, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a

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fragment of a capsular polysaccharide. A one hundred and fifty-fifth embodiment of the pharmaceutical composition of embodiment 154, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. A one hundred and fifty-sixth embodiment of the pharmaceutical composition of any one of embodiments 152-155, wherein the polypeptide is an oligopeptide. A one hundred and fifty-seventh embodiment of the pharmaceutical composition of any one of embodiments 152-156, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, or any fragment thereof. A one hundred and fifty-eighth embodiment of the pharmaceutical composition of any one of embodiments 152-157, wherein a toxin activity of the polypeptide is at least partly mitigated. A one hundred and fifty-ninth embodiment of the pharmaceutical composition of any one of embodiments 152-158, wherein the polypeptide comprises a mixture of polypeptides. A one hundred and sixtieth embodiment of the pharmaceutical composition of embodiment 159, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, any combination thereof. A one hundred and sixty-first embodiment of the pharmaceutical composition of any one of embodiments 152-160, comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38. A one hundred and sixty-second embodiment of the pharmaceutical composition of any one of embodiments 152-160, comprising a plurality of at

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least 25 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38. A one hundred and sixty-third embodiment of the pharmaceutical composition of any one of embodiments 152-160, comprising a plurality of at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B. A one hundred and sixty-fourth embodiment of the pharmaceutical composition of any one of embodiments 152-160, comprising a plurality of at least 19 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35B and 38. A one hundred and sixty-fifth embodiment of the pharmaceutical composition in unit dose form comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38. A one hundred and sixty-sixth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38. A one hundred and sixty-seventh embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A one hundred and sixty-eighth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A one hundred and sixty-ninth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A one hundred and seventieth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A one hundred and seventy-first embodiment of the

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pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A one hundred and seventy-second embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A one hundred and seventy-third embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B A one hundred and seventy-fourth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A one hundred and seventy-fifth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A one hundred and seventh-sixth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A one hundred and seventy-seventh embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A one hundred and seventy-eighth embodiment of the pharmaceutical composition of embodiment 165, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B. A one hundred and seventy-nine embodiment of the pharmaceutical composition of embodiment 165-178, wherein the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide. A one hundred and eightieth embodiment of the pharmaceutical composition of embodiment 165-178 wherein the immunogenic saccharide-polypeptide conjugate comprises a fragment of the capsular polysaccharide. A one hundred and eighty-first embodiment of the pharmaceutical composition of embodiment 180, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. A one hundred and eighty-second embodiment of the pharmaceutical composition of any one of embodiments 165-181, wherein the polypeptide is an oligopeptide. A one hundred and eighty-third embodiment of the pharmaceutical composition of any one of embodiments 165-182, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin,

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pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), and protein D from *H. influenzae*, any fragment thereof, or any combination thereof. A one hundred and eighty-fourth embodiment of the pharmaceutical composition of any one of embodiments 165-183, wherein a toxin activity of a polypeptide is at least partly mitigated. A one hundred and eighty-fifth embodiment of the pharmaceutical composition of any one of embodiments 152-184, further comprising an adjuvant. A one hundred and eighty-sixth embodiment of the pharmaceutical composition of embodiment 185, wherein the adjuvant is an aluminum-cation comprising adjuvant. A one hundred and eighty-seventh embodiment of the pharmaceutical composition of embodiment 186, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide. A one hundred and eighty-eighth embodiment of the pharmaceutical composition of any one of embodiments 152-187, wherein the pharmaceutical composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition. A one hundred and eighty-ninth embodiment of the pharmaceutical composition of any one of embodiments 152-188, further comprising a chelating agent. A one hundred and ninetieth embodiment of the pharmaceutical composition embodiment 189, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA). A one hundred and ninety-first embodiment of the pharmaceutical composition of any one of embodiments 152-190, wherein the pharmaceutical composition is in the form of a suspension. A one hundred and ninety-second embodiment of the pharmaceutical composition of any one of embodiments 152-191, wherein the pharmaceutical composition is in the form of a homogenous mixture or a heterogeneous mixture. A one hundred and ninety-third embodiment of the pharmaceutical composition of embodiment 189, wherein the pharmaceutical composition is in the form of a homogenous mixture. A one hundred and ninety-fourth embodiment of the pharmaceutical composition of any one of embodiments 152 or 161-179, which is contained in a pre-filled syringe. A one hundred and ninety-fifth embodiment of the pharmaceutical composition of any one of embodiments 152-194, further comprising a surfactant , an emulsifier, or a combination thereof. A one hundred and ninety-sixth embodiment of the pharmaceutical composition embodiment 194, comprising the

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surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. A one hundred and ninety-seventh embodiment of the pharmaceutical composition of embodiment 196, comprising the polysorbate wherein the polysorbate is polysorbate 80. A one hundred and ninety-eighth embodiment of the pharmaceutical composition of embodiment 196, comprising the polymer glycol wherein the polymer glycol is polyethylene glycol. A one hundred and ninety-nine embodiment of the pharmaceutical composition of embodiment of any one of embodiments 152-198, further comprising a buffering agent. A two hundredth embodiment of the pharmaceutical composition of embodiment 199, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof. A two hundred and first embodiment of the pharmaceutical composition of any one of embodiments 152-200, further comprising sterile water. A two hundred and second embodiment of the pharmaceutical composition of any one of embodiment 152-201, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash. A two hundred and third embodiment of the pharmaceutical composition of any one of embodiments 152-202, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years. A two hundred and fourth embodiment of the pharmaceutical composition of any one of embodiments 152-202, biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0070] A two hundred and fifth embodiment of a kit comprising of a pharmaceutical composition of any of embodiments 1-40 or 152-204 contained in a container. A two hundred and sixth embodiment of the kit of embodiment 205, further comprising instructions for use. A two hundred and seventh embodiment of the kit of any one of embodiments 205-206, wherein the container is a syringe. A two hundred and eighth embodiment of the kit of any one of

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embodiments 205-207, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

[0071] A two hundred and ninth embodiment of the composition of, or composition employed in the method of, any claim, further comprising a preservative. A two hundred and tenth embodiment of the composition of, or composition employed in the method of, any claim, further comprising an anti-fungal compound or a salt thereof. A two hundred and eleventh embodiment of the composition of, or composition employed in the method of, any claim, further comprising a salt. A two hundred and twelfth embodiment of the composition of, or composition employed in the method of, claim 211, wherein the salt is an inorganic salt. A two hundred and thirteenth embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified. A two hundred and fourteenth embodiment of the composition of, or composition employed in the method of, any claim, which is a vaccine. A two hundred and fifteenth embodiment of the method of any claim which is a method of vaccinating a subject. A two hundred and sixteenth embodiment of the method of any claim, wherein the subject is a human. A two hundred and seventeenth embodiment of the method of any claim, wherein the subject or human is a subject or human in need thereof. A two hundred and eighteenth embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition. A two hundred and nineteenth embodiment of the method of any claim, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers. A two hundred and twentieth embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using a bacteria. A two hundred and twenty-first embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using *E. coli*. A two hundred and twenty-second embodiment

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of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide. A two hundred and twenty-third embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide. A two hundred and twenty-fourth embodiment of the composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

EXAMPLES

[0072] The following examples are included to further describe some aspects of the present disclosure, and should not be used to limit the scope of the embodiments described herein.

EXAMPLE 1

Method of vaccination for adults 50 years old or older

[0073] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for adults 50 years old or older. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B . Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0074] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to an adult subject age 50 or older. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to an adult subject age 50 or older. Alternatively to four weeks later, one year later a single 0.5 of the second aqueous vaccine is given by intramuscular injection to an adult age 50 or older.

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EXAMPLE 2

Method of vaccination for children ages 2 to 17 years old

[0075] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for children ages 2 to 17 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0076] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child who is 2-17 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to a child who is 2-17 years old. Alternatively to four weeks later, one year later a single 0.5 of the second aqueous vaccine is given by intramuscular injection to a child who is 2-17 years old.

EXAMPLE 3

Method of vaccination for *S. pneumoniae* and *N. meningitidis*

[0077] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *N. meningitidis*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 20, 22F, 23A, 23B, 31, 33F, 33B, and 38 conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C capsular saccharide conjugated to CRM_{197}. Each vaccine is formulated for intramuscular injection.

[0078] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine is given by intramuscular injection.

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EXAMPLE 4

Method of vaccination for *S. pneumoniae* and *S. enterica*

[0079] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *S. enterica*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 20, 22F, 23A, 23B, 31, 33F, 33B, and 38 conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *S. enterica* subspecies enterica serovar Typhi Vi-capsular saccharide conjugated to CRM_{197}. Each aqueous vaccine is formulated for intramuscular injection.

[0080] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

EXAMPLE 5

Method of vaccination for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*

[0081] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 20, 22F, 23A, 23B, 31, 33F, 33B, and 38 conjugated to a tetanus toxoid carrier protein. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C and group Y saccharide and *H. influenzae* type b saccharide individually conjugated a tetanus toxoid carrier protein. Each aqueous vaccine is formulated for intramuscular injection.

[0082] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

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EXAMPLE 6

Composition and method of use of a vaccination for protection against *S. pneumoniae* in the United States and Canada

[0083] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in the United States and Canada. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 33F, 35F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0084] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in the United States and Canada is improved.

EXAMPLE 7

Composition and method of use of a vaccination for protection against *S. pneumoniae* in Europe, the Middle East, and North Africa

[0085] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in Europe, the Middle East, and North Africa. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0086] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in Europe, the Middle East, and North Africa is improved.

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EXAMPLE 8

Composition and method of use of a vaccination for protection against *S. pneumoniae* in the Japan and Korea

[0087] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in Japan and Korea. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0088] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in Japan and Korea is improved.

EXAMPLE 9

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0089] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0090] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 10

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0091] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. The aqueous

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vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0092] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 11

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0093] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The vaccine aqueous composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0094] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 12

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0095] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0096] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

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EXAMPLE 13

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0097] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0098] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 14

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0099] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0100] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 15

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0101] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

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[0102] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 16

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0103] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0104] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 17

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0105] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0106] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 18

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0107] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic

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saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0108] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 19

Composition and method of use of a vaccination for protection against *S. pneumoniae* in the United States and Canada

[0109] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in the United States and Canada. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0110] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in the United States and Canada is improved.

EXAMPLE 20

Composition and method of use of a vaccination for protection against *S. pneumoniae* in Europe, the Middle East, and North Africa

[0111] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in Europe, the Middle East, and North Africa. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35F. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

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[0112] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in Europe, the Middle East, and North Africa is improved.

EXAMPLE 21

Composition and method of use of a vaccination for protection against *S. pneumoniae* in the Japan and Korea

[0113] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects in Japan and Korea. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0114] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease found in Japan and Korea is improved.

EXAMPLE 22

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0115] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0116] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

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EXAMPLE 23

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0117] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0118] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 24

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0119] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0120] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 25

Composition and method of use of a vaccination for protection against *S. pneumoniae*

[0121] This example shows a vaccine composition that provides protection against *S. pneumoniae* disease. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

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[0122] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease is improved.

EXAMPLE 26

Method of making a saccharide-polypeptide conjugate pharmaceutical composition

[0123] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A capsular polysaccharide is isolated from a *S. pneumoniae* 6C serotype. The 6C capsular polysaccharide is conjugated to a CRM_{197} polypeptide. The saccharide-polypeptide conjugate is contacted with an excipient.

EXAMPLE 27

Composition of a kit with a saccharide-polypeptide conjugate

[0124] This example shows the composition of a kit with a saccharide-polypeptide conjugate. A saccharide-polypeptide conjugate in a vial or a pre-filled syringe is placed in a container. The container is further associated with instructions of use.

[0125] While preferred embodiments have been shown and described herein, it will be apparent to those skilled in the art that such embodiments are provided by way of example only. It should be understood that various alternatives

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CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition in unit dose form comprising a plurality of at least four unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide or a fragment of the capsular polysaccharide from a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of Streptococcus pneumoniae.
2. The pharmaceutical composition in unit dose form of claim 1, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.
3. The pharmaceutical composition in unit dose form of claim 1, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.
4. The pharmaceutical composition in unit dose form of claim 3, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.
5. The pharmaceutical composition in unit dose form of any one of claims 1-4, wherein at least one polypeptide of the plurality is an oligopeptide.
6. The pharmaceutical composition in unit dose form of any one of claims 1-5, wherein at least one polypeptide of the plurality comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.
7. The pharmaceutical composition in unit dose form of any one of claims 1-6, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.
8. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each

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comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

9. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

10. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

11. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

12. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

13. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

14. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

15. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

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16. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
17. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.
18. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
19. The pharmaceutical composition in unit dose form of any one of claims 1-7, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
20. The pharmaceutical composition in unit dose form of any one of claims 1-19, further comprising an adjuvant.
21. The pharmaceutical composition in unit dose form of claim 20, wherein the adjuvant is an aluminum-cation comprising adjuvant.
22. The pharmaceutical composition in unit dose form of claim 21, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.
23. The pharmaceutical composition in unit dose form of any one of claims 1-22, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.
24. The pharmaceutical composition in unit dose form of any one of claims 1-23, further comprising a chelating agent.
25. The pharmaceutical composition in unit dose form of claim 24, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

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26. The pharmaceutical composition in unit dose form of any one of claims 1-25, which is in a form of a suspension.
27. The pharmaceutical composition in unit dose form of any one of claims 1-26, which is in the form of a homogenous mixture or a heterogeneous mixture.
28. The pharmaceutical composition in unit dose form of claim 27, wherein the pharmaceutical composition is in a form of a homogenous mixture.
29. The pharmaceutical composition in unit dose form of any one of claims 1-28, which is contained in a pre-filled syringe.
30. The pharmaceutical composition in unit dose form of any one of claims 1-29, further comprising a surfactant , an emulsifier, or a combination thereof.
31. The pharmaceutical composition in unit dose form of claim 30, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.
32. The pharmaceutical composition in unit dose form of claim 31, comprising the polysorbate, wherein the polysorbate is polysorbate 80.
33. The pharmaceutical composition in unit dose form of claim 31, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.
34. The pharmaceutical composition in unit dose form of any one of claims 1-33, further comprising a buffering agent.
35. The pharmaceutical composition in unit dose form of claim 34, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.
36. The pharmaceutical composition in unit dose form of any one of claims 1-35, further comprising sterile water.
37. The pharmaceutical composition in unit dose form of any one of claims 1-36, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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38. The pharmaceutical composition in unit dose form of any one of claims 1-37, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.
39. The pharmaceutical composition in unit dose form of any one of claims 1-38, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.
40. The pharmaceutical composition in unit dose form of any one of claims 1-39, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
41. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition in unit dose form of any one of claims 1-40.
42. The method of claim 41, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.
43. The method of claim 41-42, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of Streptococcus pneumoniae conjugated to the second polypeptide.
44. The method of any one of claims 41-43, wherein the second composition comprises a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.
45. The method of any one of claims 41-43, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

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46. The method of any one claims 41-43, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.
47. The method of claim 43, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.
48. The method of claim 43, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises the fragment of the capsular polysaccharide.
49. The method of claim 48, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.
50. The method of any one of claims 41-49, wherein the second polypeptide is an oligopeptide.
51. The method any one of claims 41-48, wherein the second polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.
52. The method of any one of claims 41-51, wherein a toxin activity of the second polypeptide is at least partly mitigated.
53. The method of any one of claims 41-52, wherein the second composition further comprises an adjuvant.
54. The method of claim 53, wherein the adjuvant is an aluminum-cation comprising adjuvant.
55. The method of claim 53, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.
56. The method any one of claims 41-55, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

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57. The method of any one of claims 41-56, wherein the second composition further comprises a chelating agent.
58. The method of claim 57, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).
59. The method any one of claims 41-58, wherein the second composition is in a form of a suspension.
60. The method any one of claims 41-59, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.
61. The method any one of claims 41-60, wherein the second composition is in the form of a homogenous mixture.
62. The method any one of claims 41-61, wherein the second composition is contained in a pre-filled syringe.
63. The method any one of claims 41-62, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.
64. The method claim 63,wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.
65. The method of claim 64, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.
66. The method of claim 64, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.
67. The method any one of claims 41-66, wherein the second composition further comprises a buffering agent.
68. The method of claim 67, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.
69. The method any one of claims 41-68, wherein the second composition further comprises sterile water.

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70. The method any one of claims 41-69, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

71. The method any one of claims 41-70, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

72. The method of any one of claims 41-72, wherein the second composition is administered intramuscularly the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

73. The method of any one of claims 41-73, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

74. A method comprising:

administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition;

and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype;

wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

75. The method of claim 74, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

76. The method of claim 75, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

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77. The method of claim 76, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.
78. The method of any one of claims 74-77, wherein the first polypeptide is an oligopeptide.
79. The method of any one of claims 74-78, wherein the second polypeptide is an oligopeptide.
80. The method of any one of claims 74-79, wherein the first polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof .
81. The method of any one of claims 74-80, wherein a toxin activity of the first polypeptide is at least partly mitigated.
82. The method of any one of claims 74-81, wherein the second polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenza, any fragment thereof, or any combination thereof.
83. The method of any one of claims 74-82, wherein a toxin activity of the second polypeptide is at least partly mitigated.
84. The method of any one of claims 74-83, wherein the first serotype is from a same bacteria as the second serotype.
85. The method of any one of claims 74-84, wherein the first serotype is from a different bacteria than the second serotype.
86. The method of any one of claims 74-85, wherein the first serotype is from Streptococcus pneumoniae.

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87. The method of any one of claims 74-86, wherein the first serotype is from Neisseria meningitidis, Salmonella enterica, or Haemophilus influenzae.
88. The method of any one of claims 74-87, wherein the second serotype is from Streptococcus pneumoniae.
89. The method of any one of claims 74-88, wherein the second bacteria is from Neisseria meningitidis, Salmonella enterica, or Haemophilus influenzae.
90. The method of any one of claims 74-89, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from Streptococcus pneumoniae, and the second serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F,24B, 31, 33F, 35F, 35B, or 38 from Streptococcus pneumoniae.
91. The method of any one of claims 74-89, wherein the first serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from Streptococcus pneumoniae, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from Streptococcus pneumoniae.
92. The method of any one of claims 74-91, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.
93. The method of claim 92, wherein the adjuvant is an aluminum-cation comprising adjuvant.
94. The method of claim 93, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.
95. The method of any one of claims 74-94, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.
96. The method of any one of claims 74-95, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.
97. The method of claim 96, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).
98. The method of any one of claims 74-97, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

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99. The method of any one of claims 74-98, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

100. The method of any one of claims 74-99, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

101. The method of any one of claims 74-100, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

102. The method of any one of claims 74-101, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

103. The method of claim 102, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

104. The method of claim 103, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

105. The method claim 103, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

106. The method of any one of claims 74-105, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

107. The method of claim 106, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

108. The method of any one of claims 74-107, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

109. The method of any one of claims 74-108, wherein when the first immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the

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plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

110. The method of any one of claims 74-109, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

111. The method of any one of claims 74-110, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

112. The method of any one of claims 74-111, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

113. The method of claim 112, wherein the adjuvant is an aluminum-cation comprising adjuvant.

114. The method of claim 112, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

115. The method of any one of claims 74-114, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

116. The method of any one of claims 74-115, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

117. The method of claim 116, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

118. The method of any one of claims 74-117, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

119. The method of any one of claims 74-118, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

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120. The method of claim 119, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

121. The method of any one of claims 74-120, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

122. The method of any one of claims 74-121, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

123. The method of claim 122, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

124. The method of claim 123, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

125. The method of claim 123, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

126. The method of any one of claims 74-125, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

127. The method of claim 126, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

128. The method of any one of claims 74-127, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

129. The method of any one of claims 74-128, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

130. The method of any one of claims 74-129, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human,

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the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

131. The method of any one of claims 74-130, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure

132. A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 1-19 with an excipient, an adjuvant, or any combination thereof.

133. The method of claim 132, wherein the composition is injectable or intranasally administrable.

134. The method claim 132, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

135. The method of any one of claims 132-134, wherein the composition is in the form of an intramuscularly injectable composition.

136. The method of any one of claims 132-135, further comprising contacting the composition with a chelating agent.

137. The method of claim 136, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

138. The method of any one of claims 132-137, wherein the composition is in a form of a suspension.

139. The method of any one of claims 132-138, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

140. The method of any one of claims 139, wherein the composition is in a form of a homogenous mixture.

141. The method of any one of claims 132-140, wherein the composition is contained in a pre-filled syringe.

142. The method of any one of claims 132-141, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

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143. The method of claim 142, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

144. The method of claim 143, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

145. The method of claim 143, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

146. The method of any one of claims 132-145, further comprising contacting the composition with a buffering agent.

147. The method of claim 146, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

148. The method of any one of claims 132-147, further comprising contacting the composition with sterile water.

149. The method of any one of claims 132-148, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

150. The method of any one of claims 132-149, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

151. The method of any one of claims 132-150, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

152. A pharmaceutical composition in unit dose form comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V,

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14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38.

153. The pharmaceutical composition in unit dose form of claim 152, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a capsular polysaccharide.

154. The pharmaceutical composition in unit dose form of claim 152, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a fragment of a capsular polysaccharide.

155. The pharmaceutical composition in unit dose form of claim 154, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

156. The pharmaceutical composition in unit dose form of any one of claims 152-155, wherein the polypeptide is an oligopeptide.

157. The pharmaceutical composition in unit dose form of any one of claims 152-156, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, or any fragment thereof.

158. The pharmaceutical composition in unit dose form of any one of claims 152-157, wherein a toxin activity of the polypeptide is at least partly mitigated.

159. The pharmaceutical composition in unit dose form of any one of claims 152-158, wherein the polypeptide comprises a mixture of polypeptides.

160. The pharmaceutical composition in unit dose form of claim 159, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N.

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meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, any combination thereof.

161. The pharmaceutical composition in unit dose form of any one of claims 152-160, comprising a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38.

162. The pharmaceutical composition in unit dose form of any one of claims 152-160, comprising a plurality of at least 25 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.

163. The pharmaceutical composition in unit dose form of any one of claims 152-160, comprising a plurality of at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B.

164. The pharmaceutical composition in unit dose form of any one of claims 152-160, comprising a plurality of at least 19 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35B and 38.

165. A pharmaceutical composition in unit dose form comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38.

166. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.

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167. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

168. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B.

169. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

170. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

171. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

172. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

173. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

174. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

175. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

176. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B.

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177. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F.

178. The pharmaceutical composition in unit dose form of claim 165, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.

179. The pharmaceutical composition in unit dose form of claim 165-178, wherein the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

180. The pharmaceutical composition in unit dose form of claim 165-178 wherein the immunogenic saccharide-polypeptide conjugate comprises a fragment of the capsular polysaccharide.

181. The pharmaceutical composition in unit dose form of claim 180, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

182. The pharmaceutical composition in unit dose form of any one of claims 165-181, wherein the polypeptide is an oligopeptide.

183. The pharmaceutical composition in unit dose form of any one of claims 165-182, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable H. influenzae P4 protein, non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, the purified protein derivative of tuberculin(PPD), and protein D from H. influenzae, any fragment thereof, or any combination thereof.

184. The pharmaceutical composition in unit dose form of any one of claims 165-183, wherein a toxin activity of a polypeptide is at least partly mitigated.

185. The pharmaceutical composition in unit dose form of any one of claims 152-184, further comprising an adjuvant.

186. The pharmaceutical composition in unit dose form of claim 185, wherein the adjuvant is an aluminum-cation comprising adjuvant.

187. The pharmaceutical composition in unit dose form of claim 186, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

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188. The pharmaceutical composition in unit dose form of any one of claims 152-187, wherein the pharmaceutical composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

189. The pharmaceutical composition in unit dose form of any one of claims 152-188, further comprising a chelating agent.

190. The pharmaceutical composition in unit dose form of claim 189, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

191. The pharmaceutical composition in unit dose form of any one of claims 152-190, wherein the pharmaceutical composition is in the form of a suspension.

192. The pharmaceutical composition in unit dose form of any one of claims 152-191, wherein the pharmaceutical composition is in the form of a homogenous mixture or a heterogeneous mixture.

193. The pharmaceutical composition in unit dose form of claim 189, wherein the pharmaceutical composition is in the form of a homogenous mixture.

194. The pharmaceutical composition in unit dose form of any one of claims 152 or 165-178, which is contained in a pre-filled syringe.

195. The pharmaceutical composition in unit dose form of any one of claims 152-194, further comprising a surfactant , an emulsifier, or a combination thereof.

196. The pharmaceutical composition in unit dose form of claim 195, comprising the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

197. The pharmaceutical composition in unit dose form of claim 196, comprising the polysorbate wherein the polysorbate is polysorbate 80.

198. The pharmaceutical composition in unit dose form of claim 197, comprising the polymer glycol wherein the polymer glycol is polyethylene glycol.

199. The pharmaceutical composition in unit dose form of claim of any one of claims 152-198, further comprising a buffering agent.

200. The pharmaceutical composition in unit dose form of claim 199, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering

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agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

201. The pharmaceutical composition in unit dose form of any one of claims 152-200, further comprising sterile water.

202. The pharmaceutical composition in unit dose form of any one of claim 152-201, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

203. The pharmaceutical composition in unit dose form of any one of claims 152-202, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

204. The pharmaceutical composition in unit dose form of any one of claims 152-202, biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

205. A kit comprising of a pharmaceutical composition of any of claims 1-40 or 152-204 contained in a container.

206. The kit of claim 205, further comprising instructions for use.

207. The kit of any one of claims 205-206, wherein the container is a syringe.

208. The kit of any one of claims 205-207, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

209. The composition of, or composition employed in the method of, any claim, further comprising a preservative.

210. The composition of, or composition employed in the method of, any claim, further comprising an anti-fungal compound or a salt thereof.

211. The composition of, or composition employed in the method of, any claim, further comprising a salt.

212. The composition of, or composition employed in the method of, claim 211, wherein the salt is an inorganic salt.

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213. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

214. The composition of, or composition employed in the method of, any claim, which is a vaccine.

215. The method of any claim which is a method of vaccinating a subject.

216. The method of any claim, wherein the subject is a human.

217. The method of any claim, wherein the subject or human is a subject or human in need thereof.

218. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

219. The method of any claim, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

220. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using a bacteria.

221. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using E. coli.

222. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

223. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

224. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

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225. A pharmaceutical composition in unit dose form comprising a plurality of immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least four serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of Streptococcus pneumoniae.

226. The pharmaceutical composition in unit dose form of claim 225, wherein the plurality of immunogenic saccharide-polypeptide conjugates comprises the capsular polysaccharides.

227. The pharmaceutical composition in unit dose form of claim 225, wherein the plurality of immunogenic saccharide-polypeptide conjugates comprises fragments of the capsular polysaccharide.

228. The pharmaceutical composition in unit dose form of claim 226, wherein fragments of the capsular polysaccharide comprise a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

229. The pharmaceutical composition in unit dose form of any one of claims 225-228, wherein at least one polypeptide of the plurality is an oligopeptide.

230. The pharmaceutical composition in unit dose form of any one of claims 225-229, wherein at least one polypeptide of the plurality comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

231. The pharmaceutical composition in unit dose form of any one of claims 225-230, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

232. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

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233. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

234. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

235. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

236. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

237. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

238. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

239. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

240. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide conjugates

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each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

241. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

242. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

243. The pharmaceutical composition in unit dose form of any one of claims 225-231, comprising a plurality of at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

244. The pharmaceutical composition in unit dose form of any one of claims 225-243, further comprising an adjuvant.

245. The pharmaceutical composition in unit dose form of claim 244, wherein the adjuvant is an aluminum-cation comprising adjuvant.

246. The pharmaceutical composition in unit dose form of claim 245, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

247. The pharmaceutical composition in unit dose form of any one of claims 225-246, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

248. The pharmaceutical composition in unit dose form of any one of claims 225-247, further comprising a chelating agent.

249. The pharmaceutical composition in unit dose form of claim 248, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

250. The pharmaceutical composition in unit dose form of any one of claims 225-249, which is in a form of a suspension.

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251. The pharmaceutical composition in unit dose form of any one of claims 225-250, which is in the form of a homogenous mixture or a heterogeneous mixture.

252. The pharmaceutical composition in unit dose form of claim 251, wherein the pharmaceutical composition is in a form of a homogenous mixture.

253. The pharmaceutical composition in unit dose form of any one of claims 225-252, which is contained in a pre-filled syringe.

254. The pharmaceutical composition in unit dose form of any one of claims 225-253, further comprising a surfactant , an emulsifier, or a combination thereof.

255. The pharmaceutical composition in unit dose form of claim 254, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

256. The pharmaceutical composition in unit dose form of claim 255, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

257. The pharmaceutical composition in unit dose form of claim 255, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

258. The pharmaceutical composition in unit dose form of any one of claims 225-257, further comprising a buffering agent.

259. The pharmaceutical composition in unit dose form of claim 258, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

260. The pharmaceutical composition in unit dose form of any one of claims 225-259, further comprising sterile water.

261. The pharmaceutical composition in unit dose form of any one of claims 225-260, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

262. The pharmaceutical composition in unit dose form of any one of claims 225-261, wherein when the pharmaceutical composition is administered intramuscularly to a human, the

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pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

263. The pharmaceutical composition in unit dose form of any one of claims 225-262, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

264. The pharmaceutical composition in unit dose form of any one of claims 225-263, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

265. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition in unit dose form of any one of claims 225-263.

266. The method of claim 265, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

267. The method any one of claims 265-266, wherein the second polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

268. The method of any one of claims 265-266, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

269. A pharmaceutical composition in unit dose form comprising a plurality of immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least 13 serotype selected from the group consisting of

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1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 of S. pneumoniae.

270. The pharmaceutical composition in unit dose form of claim 269, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, or any fragment thereof.

271. The pharmaceutical composition in unit dose form of any one of claims 269-270, wherein a toxin activity of the polypeptide is at least partly mitigated.

272. The pharmaceutical composition in unit dose form of any one of claims 269-271, wherein the polypeptide comprises a mixture of polypeptides.

273. The pharmaceutical composition in unit dose form of claim 272, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, any combination thereof.

274. The pharmaceutical composition in unit dose form of claim 273, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

275. A kit comprising of a pharmaceutical composition of any of claims 225-63 or 269-274 contained in a container.

276. The kit of claim 275, further comprising instructions for use.

277. The kit of any one of claims 275-276, wherein the container is a syringe.

278. The kit of any one of claims 275-277, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

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279. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

280. A pharmaceutical composition comprising at least four unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide or a fragment of the capsular polysaccharide from a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of *Streptococcus pneumoniae*.

281. The pharmaceutical composition of claim 280, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

282. The pharmaceutical composition of claim 280, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.

283. The pharmaceutical composition of claim 282, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

284. The pharmaceutical composition of any one of claims 280-283, wherein at least one polypeptide of the plurality is an oligopeptide.

285. The pharmaceutical composition of any one of claims 280-284, wherein at least one polypeptide of the plurality comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof.

286. The pharmaceutical composition of any one of claims 280-285, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

287. The pharmaceutical composition of any one of claims 280-286, comprising at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype

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selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

288. The pharmaceutical composition of any one of claims 280-286, comprising at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

289. The pharmaceutical composition of any one of claims 280-286, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

290. The pharmaceutical composition of any one of claims 280-286, comprising at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

291. The pharmaceutical composition of any one of claims 280-286, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

292. The pharmaceutical composition of any one of claims 280-286, comprising at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

293. The pharmaceutical composition of any one of claims 280-286, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

294. The pharmaceutical composition of any one of claims 280-286, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

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295. The pharmaceutical composition of any one of claims 280-286, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

296. The pharmaceutical composition of any one of claims 280-286, comprising at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

297. The pharmaceutical composition of any one of claims 280-286, comprising at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

298. The pharmaceutical composition of any one of claims 280-286, comprising at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

299. The pharmaceutical composition of any one of claims 280-298, further comprising an adjuvant.

300. The pharmaceutical composition of claim 299, wherein the adjuvant is an aluminum-cation comprising adjuvant.

301. The pharmaceutical composition of claim 300, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

302. The pharmaceutical composition of any one of claims 280-301, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

303. The pharmaceutical composition of any one of claims 280-302, further comprising a chelating agent.

304. The pharmaceutical composition of claim 280-303, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

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305. The pharmaceutical composition of any one of claims 280-304, which is in a form of a suspension.

306. The pharmaceutical composition of any one of claims 280-305, which is in the form of a homogenous mixture or a heterogeneous mixture.

307. The pharmaceutical composition of claim 306, wherein the pharmaceutical composition is in a form of a homogenous mixture.

308. The pharmaceutical composition of any one of claims 280-307, which is contained in a pre-filled syringe.

309. The pharmaceutical composition of any one of claims 280-308, further comprising a surfactant, an emulsifier, or a combination thereof.

310. The pharmaceutical composition of claim 309, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

311. The pharmaceutical composition of claim 310, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

312. The pharmaceutical composition of claim 311, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

313. The pharmaceutical composition of any one of claims 280-312, further comprising a buffering agent.

314. The pharmaceutical composition of claim 313, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

315. The pharmaceutical composition of any one of claims 280-314, further comprising sterile water.

316. The pharmaceutical composition of any one of claims 280-315, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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317. The pharmaceutical composition of any one of claims 280-316, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

318. The pharmaceutical composition of any one of claims 280-317, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

319. The pharmaceutical composition of any one of claims 280-318, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

320. The pharmaceutical composition of any one of claims 280-319 in unit dose form.

321. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 280-320.

322. The method of claim 321, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

323. The method of claim 321-322, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

324. The method of any one of claims 321-323, wherein the second composition comprises at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

325. The method of any one of claims 321-324, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

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326. The method of any one claims 321-323, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.

327. The method of claim 323, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.

328. The method of claim 323, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises the fragment of the capsular polysaccharide.

329. The method of claim 328, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

330. The method of any one of claims 321-329, wherein the second polypeptide is an oligopeptide.

331. The method any one of claims 321-328, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof.

332. The method of any one of claims 321-331, wherein a toxin activity of the second polypeptide is at least partly mitigated.

333. The method of any one of claims 321-332, wherein the second composition further comprises an adjuvant.

334. The method of claim 333, wherein the adjuvant is an aluminum-cation comprising adjuvant.

335. The method of claim 333, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

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336. The method any one of claims 321-335, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

337. The method of any one of claims 321-335, wherein the second composition further comprises a chelating agent.

338. The method of claim 337, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

339. The method any one of claims 321-338, wherein the second composition is in a form of a suspension.

340. The method any one of claims 321-339, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.

341. The method any one of claims 321-340, wherein the second composition is in the form of a homogenous mixture.

342. The method any one of claims 321-341, wherein the second composition is contained in a pre-filled syringe.

343. The method any one of claims 321-342, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.

344. The method claim 343,wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

345. The method of claim 344, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.

346. The method of claim 345, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

347. The method any one of claims 321-346, wherein the second composition further comprises a buffering agent.

348. The method of claim 347, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

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349. The method any one of claims 321-348, wherein the second composition further comprises sterile water.

350. The method any one of claims 321-349, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

351. The method any one of claims 321-350, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

352. The method of any one of claims 321-351, wherein the second composition is administered intramuscularly the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

353. The method of any one of claims 321-352, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

354. The method of any one of claims 321-353, wherein the pharmaceutical composition is in unit dose form.

355. A method comprising:

a) administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered;

b) wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition;

c) and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype;

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d) wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

356. The method of claim 355, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

357. The method of claim 356, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

358. The method of claim 357, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

359. The method of any one of claims 355-358, wherein the first polypeptide is an oligopeptide.

360. The method of any one of claims 355-359, wherein the second polypeptide is an oligopeptide.

361. The method of any one of claims 355-360, wherein the first polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof .

362. The method of any one of claims 355-361, wherein a toxin activity of the first polypeptide is at least partly mitigated.

363. The method of any one of claims 355-362, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N.*

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meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenza*, any fragment thereof, or any combination thereof.

364. The method of any one of claims 355-363, wherein a toxin activity of the second polypeptide is at least partly mitigated.

365. The method of any one of claims 355-364, wherein the first serotype is from a same bacteria as the second serotype.

366. The method of any one of claims 355-365, wherein the first serotype is from a different bacteria than the second serotype.

367. The method of any one of claims 355-366, wherein the first serotype is from *Streptococcus pneumoniae*.

368. The method of any one of claims 355-367, wherein the first serotype is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

369. The method of any one of claims 355-368, wherein the second serotype is from *Streptococcus pneumoniae*.

370. The method of any one of claims 355-369, wherein the second bacteria is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

371. The method of any one of claims 355-370, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F,24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

372. The method of any one of claims 355-371, wherein the first serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

373. The method of any one of claims 355-372, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

374. The method of claim 373, wherein the adjuvant is an aluminum-cation comprising adjuvant.

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375. The method of claim 374, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

376. The method of any one of claims 355-375, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

377. The method of any one of claims 355-376, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

378. The method of claim 377, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

379. The method of any one of claims 355-378, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

380. The method of any one of claims 355-379, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

381. The method of any one of claims 355-380, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

382. The method of any one of claims 355-381, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

383. The method of any one of claims 355-382, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

384. The method of claim 383, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

385. The method of claim 384, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

386. The method claim 385, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

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387. The method of any one of claims 355-386, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

388. The method of claim 387, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

389. The method of any one of claims 355-388, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

390. The method of any one of claims 355-389, wherein when the first immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

391. The method of any one of claims 355-390, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

392. The method of any one of claims 355-391, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

393. The method of any one of claims 355-392, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

394. The method of claim 393, wherein the adjuvant is an aluminum-cation comprising adjuvant.

395. The method of claim 393, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

396. The method of any one of claims 355-395, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition,

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intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

397. The method of any one of claims 355-396, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

398. The method of claim 397, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

399. The method of any one of claims 355-398, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

400. The method of any one of claims 355-399, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

401. The method of claim 400, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

402. The method of any one of claims 355-401, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

403. The method of any one of claims 355-402, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

404. The method of claim 403, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

405. The method of claim 404, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

406. The method of claim 404, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

407. The method of any one of claims 355-406, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

408. The method of claim 407, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a

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sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

409. The method of any one of claims 355-408, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

410. The method of any one of claims 355-409, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

411. The method of any one of claims 355-410, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

412. The method of any one of claims 355-411, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

413. The method of any one of claims 355-412, wherein the first immunogenic saccharide-polypeptide conjugate composition is in unit dose form.

414. The method of any one of claims 355-413, whereint the second immunogenic saccharide-polypeptide conjugate composition is in unit dose form.

415. A method of making a composition comprising:

a) contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 280-298 with an excipient, an adjuvant, or any combination thereof.

416. The method of claim 415, wherein the composition is injectable or intranasally administrable.

417. The method claim 415, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

418. The method of any one of claims 415-417, wherein the composition is in the form of an intramuscularly injectable composition.

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419. The method of any one of claims 415-418, further comprising contacting the composition with a chelating agent.

420. The method of claim 419, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

421. The method of any one of claims 415-420, wherein the composition is in a form of a suspension.

422. The method of any one of claims 415-421, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

423. The method of any one of claims 422, wherein the composition is in a form of a homogenous mixture.

424. The method of any one of claims 415-423, wherein the composition is contained in a pre-filled syringe.

425. The method of any one of claims 415-424, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

426. The method of claim 425, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

427. The method of claim 426, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

428. The method of claim 426, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

429. The method of any one of claims 415-428, further comprising contacting the composition with a buffering agent.

430. The method of claim 429, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

431. The method of any one of claims 415-430, further comprising contacting the composition with sterile water.

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432. The method of any one of claims 415-431, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

433. The method of any one of claims 415-432, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

434. The method of any one of claims 415-433, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

435. A pharmaceutical composition comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38; each conjugated to a polypeptide.

436. The pharmaceutical composition of claim 435, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a capsular polysaccharide.

437. The pharmaceutical composition of claim 435, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a fragment of a capsular polysaccharide.

438. The pharmaceutical composition of claim 437, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

439. The pharmaceutical composition of any one of claims 435-438, wherein the polypeptide is an oligopeptide.

440. The pharmaceutical composition of any one of claims 435-439, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from

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N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, or any fragment thereof.

441. The pharmaceutical composition of any one of claims 435-440, wherein a toxin activity of the polypeptide is at least partly mitigated.

442. The pharmaceutical composition of any one of claims 435-441, wherein the polypeptide comprises a mixture of polypeptides.

443. The pharmaceutical composition of claim 442, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, any combination thereof.

444. The pharmaceutical composition of any one of claims 435-443, comprising at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38.

445. The pharmaceutical composition of any one of claims 435-443, comprising at least 25 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.

446. The pharmaceutical composition of any one of claims 435-443, comprising at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B.

447. The pharmaceutical composition of any one of claims 435-443, comprising at least 19 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35B and 38.

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448. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising a unique capsular polysaccharide serotype, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38; each conjugated to a polypeptide.

449. The pharmaceutical composition of claim 448, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.

450. The pharmaceutical composition of claim 448, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

451. The pharmaceutical composition of claim 448, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B.

452. The pharmaceutical composition of claim 448, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

453. The pharmaceutical composition of claim 448, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

454. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

455. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

456. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

457. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

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458. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

459. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B.

460. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F.

461. The pharmaceutical composition of claim 448, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.

462. The pharmaceutical composition of claim 448-461, wherein the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

463. The pharmaceutical composition of claim 448-461 wherein the immunogenic saccharide-polypeptide conjugate comprises a fragment of the capsular polysaccharide.

464. The pharmaceutical composition of claim 463, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

465. The pharmaceutical composition of any one of claims 448-464, wherein the polypeptide is an oligopeptide.

466. The pharmaceutical composition of any one of claims 448-465, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), and protein D from *H. influenzae*, any fragment thereof, or any combination thereof.

467. The pharmaceutical composition of any one of claims 448-466, wherein a toxin activity of a polypeptide is at least partly mitigated.

468. The pharmaceutical composition of any one of claims 435-467, further comprising an adjuvant.

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469. The pharmaceutical composition of claim 468, wherein the adjuvant is an aluminum-cation comprising adjuvant.

470. The pharmaceutical composition of claim 469, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

471. The pharmaceutical composition of any one of claims 435-470, wherein the pharmaceutical composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

472. The pharmaceutical composition of any one of claims 435-471, further comprising a chelating agent.

473. The pharmaceutical composition of claim 472, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

474. The pharmaceutical composition of any one of claims 435-473, wherein the pharmaceutical composition is in the form of a suspension.

475. The pharmaceutical composition of any one of claims 435-474, wherein the pharmaceutical composition is in the form of a homogenous mixture or a heterogeneous mixture.

476. The pharmaceutical composition of claim 472, wherein the pharmaceutical composition is in the form of a homogenous mixture.

477. The pharmaceutical composition of any one of claims 435 or 448-461, which is contained in a pre-filled syringe.

478. The pharmaceutical composition of any one of claims 435-468, further comprising a surfactant , an emulsifier, or a combination thereof.

479. The pharmaceutical composition of claim 478, comprising the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

480. The pharmaceutical composition of claim 479, comprising the polysorbate wherein the polysorbate is polysorbate 80.

481. The pharmaceutical composition of claim 480, comprising the polymer glycol wherein the polymer glycol is polyethylene glycol.

482. The pharmaceutical composition of claim of any one of claims 435-481, further comprising a buffering agent.

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483. The pharmaceutical composition of claim 482, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

484. The pharmaceutical composition of any one of claims 435-483, further comprising sterile water.

485. The pharmaceutical composition of any one of claim 435-484, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

486. The pharmaceutical composition of any one of claims 435-485, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

487. The pharmaceutical composition of any one of claims 435-486, biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

488. The pharmaceutical composition of any of claims 435-487 in unit dose form.

489. A kit comprising of a pharmaceutical composition of any of claims 280-320 or 435-488 contained in a container.

490. The kit of claim 489, further comprising instructions for use.

491. The kit of any one of claims 489-490, wherein the container is a syringe.

492. The kit of any one of claims 489-491, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

493. The composition of, or composition employed in the method of, any claim, further comprising a preservative.

494. The composition of, or composition employed in the method of, any claim, further comprising an anti-fungal compound or a salt thereof.

495. The composition of, or composition employed in the method of, any claim, further comprising a salt.

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496. The composition of, or composition employed in the method of, claim 495, wherein the salt is an inorganic salt.

497. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

498. The composition of, or composition employed in the method of, any claim, which is a vaccine.

499. The method of any claim which is a method of vaccinating a subject.

500. The method of any claim, wherein the subject is a human.

501. The method of any claim, wherein the subject or human is a subject or human in need thereof.

502. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

503. The method of any claim, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

504. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using a bacteria.

505. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using *E. coli*.

506. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

507. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

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508. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

509. A pharmaceutical composition comprising immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least four serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of *Streptococcus pneumoniae*; each conjugated to a polypeptide; each conjugated to a polypeptide.

510. The pharmaceutical composition of claim 509, wherein immunogenic saccharide-polypeptide conjugates comprise the capsular polysaccharides.

511. The pharmaceutical composition of claim 509, wherein immunogenic saccharide-polypeptide conjugates comprise fragments of the capsular polysaccharide.

512. The pharmaceutical composition of claim 510, wherein fragments of the capsular polysaccharide comprise a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

513. The pharmaceutical composition of any one of claims 509-512, wherein at least one polypeptide is an oligopeptide.

514. The pharmaceutical composition of any one of claims 509-513, wherein at least one polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof.

515. The pharmaceutical composition of any one of claims 509-514, wherein a toxin activity of at least one of the polypeptide is at least partly mitigated.

516. The pharmaceutical composition of any one of claims 509-514, comprising at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype

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selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

517. The pharmaceutical composition of any one of claims 509-514, comprising at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

518. The pharmaceutical composition of any one of claims 509-514, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

519. The pharmaceutical composition of any one of claims 509-514, comprising at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

520. The pharmaceutical composition of any one of claims 509-514, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

521. The pharmaceutical composition of any one of claims 509-514, comprising at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

522. The pharmaceutical composition of any one of claims 509-514, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

523. The pharmaceutical composition of any one of claims 509-514, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

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524. The pharmaceutical composition of any one of claims 509-514, comprising at least 13 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

525. The pharmaceutical composition of any one of claims 509-514, comprising at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

526. The pharmaceutical composition of any one of claims 509-514, comprising at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

527. The pharmaceutical composition of any one of claims 509-514, comprising at least 11 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

528. The pharmaceutical composition of any one of claims 509-527, further comprising an adjuvant.

529. The pharmaceutical composition of claim 528, wherein the adjuvant is an aluminum-cation comprising adjuvant.

530. The pharmaceutical composition of claim 528, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

531. The pharmaceutical composition of any one of claims 509-530, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

532. The pharmaceutical composition of any one of claims 509-531, further comprising a chelating agent.

533. The pharmaceutical composition of claim 532, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

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534. The pharmaceutical composition of any one of claims 509-533, which is in a form of a suspension.

535. The pharmaceutical composition of any one of claims 509-533, which is in the form of a homogenous mixture or a heterogeneous mixture.

536. The pharmaceutical composition of claim 535, wherein the pharmaceutical composition is in a form of a homogenous mixture.

537. The pharmaceutical composition of any one of claims 509-536, which is contained in a pre-filled syringe.

538. The pharmaceutical composition of any one of claims 509-537, further comprising a surfactant , an emulsifier, or a combination thereof.

539. The pharmaceutical composition of claim 538, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

540. The pharmaceutical composition of claim 539, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

541. The pharmaceutical composition of claim 539, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

542. The pharmaceutical composition of any one of claims 509-541, further comprising a buffering agent.

543. The pharmaceutical composition of claim 542, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

544. The pharmaceutical composition of any one of claims 509-543, further comprising sterile water.

545. The pharmaceutical composition of any one of claims 509-544, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

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546. The pharmaceutical composition of any one of claims 509-545, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

547. The pharmaceutical composition form of any one of claims 509-546, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

548. The pharmaceutical composition of any one of claims 509-547, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

549. The pharmaceutical composition of any one of claims 509-548 in unit dose form.

550. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 509-549.

551. The method of claim 550, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

552. The method any one of claims 550-551, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof.

553. The method of any one of claims 550-552, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

554. A pharmaceutical composition comprising immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least 13 serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F,

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9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 of *S. pneumoniae*; each conjugated to a polypeptide.

555. The pharmaceutical composition of claim 554, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, or any fragment thereof.

556. The pharmaceutical composition of any one of claims 554-555, wherein a toxin activity of the polypeptide is at least partly mitigated.

557. The pharmaceutical composition of any one of claims 554-556, wherein the polypeptide comprises a mixture of polypeptides.

558. The pharmaceutical composition of claim 557, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, any combination thereof.

559. The pharmaceutical composition of any one of claims 554-558, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

560. The pharmaceutical composition of any one of claims 554-559 in unit dose form.

561. A kit comprising of a pharmaceutical composition of any of claims 509-549 or 554-560 contained in a container.

562. The kit of claim 561, further comprising instructions for use.

563. The kit of any one of claims 561-562, wherein the container is a syringe.

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564. The kit of any one of claims 561-563, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

565. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

566. A pharmaceutical composition comprising at least four immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide or a fragment of the capsular polysaccharide from a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of Streptococcus pneumoniae.

567. The pharmaceutical composition of claim 566, wherein at least one of the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

568. The pharmaceutical composition of claim 566, wherein at least one of the immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.

569. The pharmaceutical composition of claim 569, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

570. The pharmaceutical composition of any one of claims 566-569, wherein at least one polypeptide of the plurality is an oligopeptide.

571. The pharmaceutical composition of any one of claims 566-570, wherein at least one polypeptide of the plurality comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

572. The pharmaceutical composition of any one of claims 566-571, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

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573. The pharmaceutical composition of any one of claims 566-572, comprising at least 9 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

574. The pharmaceutical composition of any one of claims 566-572, comprising at least 10 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

575. The pharmaceutical composition of any one of claims 566-572, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

576. The pharmaceutical composition of any one of claims 566-572, comprising at least 14 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

577. The pharmaceutical composition of any one of claims 566-572, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

578. The pharmaceutical composition of any one of claims 566-572, comprising at least 12 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

579. The pharmaceutical composition of any one of claims 566-572, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

580. The pharmaceutical composition of any one of claims 566-572, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

581. The pharmaceutical composition of any one of claims 566-572, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

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582. The pharmaceutical composition of any one of claims 566-572, comprising at least 12 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

583. The pharmaceutical composition of any one of claims 566-572, comprising at least 11 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

584. The pharmaceutical composition of any one of claims 566-572, comprising at least 11 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

585. The pharmaceutical composition of any one of claims 566-584, further comprising an adjuvant.

586. The pharmaceutical composition of claim 585, wherein the adjuvant is an aluminum-cation comprising adjuvant.

587. The pharmaceutical composition of claim 586, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

588. The pharmaceutical composition of any one of claims 566-587, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

589. The pharmaceutical composition of any one of claims 566-588, further comprising a chelating agent.

590. The pharmaceutical composition of claim 589, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

591. The pharmaceutical composition of any one of claims 566-590, which is in a form of a suspension.

592. The pharmaceutical composition of any one of claims 566-591, which is in the form of a homogenous mixture or a heterogeneous mixture.

593. The pharmaceutical composition of claim 592, wherein the pharmaceutical composition is in a form of a homogenous mixture.

594. The pharmaceutical composition of any one of claims 566-593, which is contained in a pre-filled syringe.

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595. The pharmaceutical composition of any one of claims 566-593, further comprising a surfactant, an emulsifier, or a combination thereof.

596. The pharmaceutical composition of claim 595, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

597. The pharmaceutical composition of claim 596, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

598. The pharmaceutical composition of claim 596, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

599. The pharmaceutical composition of any one of claims 566-598, further comprising a buffering agent.

600. The pharmaceutical composition of claim 599, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

601. The pharmaceutical composition of any one of claims 566-600, further comprising sterile water.

602. The pharmaceutical composition of any one of claims 566-601, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

603. The pharmaceutical composition of any one of claims 566-602, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

604. The pharmaceutical composition of any one of claims 566-603, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

605. The pharmaceutical composition of any one of claims 566-604, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

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606. The pharmaceutical composition of any one of claims 566-605 in unit dose form.

607. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 566-606.

608. The method of claim 42, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

609. The method of claim 607-608, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of Streptococcus pneumoniae conjugated to the second polypeptide.

610. The method of any one of claims 607-609, wherein the second composition comprises at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

611. The method of any one of claims 607-610, wherein the second composition comprises at least 1 immunogenic saccharide-polypeptide conjugate comprising a serotype selected from the group consisting of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

612. The method of any one claims 607-611, wherein the second composition comprising at least 1 immunogenic saccharide-polypeptide conjugate comprising a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.

613. The method of claim 612, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.

614. The method of claim 612, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises the fragment of the capsular polysaccharide.

615. The method of claim 614, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

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616. The method of any one of claims 607-615, wherein the second polypeptide is an oligopeptide.

617. The method any one of claims 607-616, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

618. The method of any one of claims 607-617, wherein a toxin activity of the second polypeptide is at least partly mitigated.

619. The method of any one of claims 607-618, wherein the second composition further comprises an adjuvant.

620. The method of claim 619, wherein the adjuvant is an aluminum-cation comprising adjuvant.

621. The method of claim 619, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

622. The method any one of claims 607-621, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

623. The method of any one of claims 607-622, wherein the second composition further comprises a chelating agent.

624. The method of claim 623, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

625. The method any one of claims 607-624, wherein the second composition is in a form of a suspension.

626. The method any one of claims 607-625, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.

627. The method any one of claims 607-626, wherein the second composition is in the form of a homogenous mixture.

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628. The method any one of claims 607-627, wherein the second composition is contained in a pre-filled syringe.

629. The method any one of claims 607-628, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.

630. The method claim 629,wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

631. The method of claim 630, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.

632. The method of claim 630, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

633. The method any one of claims 607-632, wherein the second composition further comprises a buffering agent.

634. The method of claim 633, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

635. The method any one of claims 607-634, wherein the second composition further comprises sterile water.

636. The method any one of claims 607-635, wherein when the second composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

637. The method any one of claims 607-636, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

638. The method of any one of claims 607-637, wherein the second composition is administered intramuscularly the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

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639. The method of any one of claims 607-638, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

640. The method of any one of claims 607-369, wherein the pharmaceutical composition is in unit dose form.

641. A method comprising:

a. administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered;

b. wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition;

c. and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype;

d. wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

642. The method of claim 641, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

643. The method of claim 641, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

644. The method of claim 643, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

645. The method of any one of claims 641-644, wherein the first polypeptide is an oligopeptide.

646. The method of any one of claims 641-645, wherein the second polypeptide is an oligopeptide.

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647. The method of any one of claims 641-646, wherein the first polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof .

648. The method of any one of claims 641-647, wherein a toxin activity of the first polypeptide is at least partly mitigated.

649. The method of any one of claims 641-648, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenza, any fragment thereof, or any combination thereof.

650. The method of any one of claims 641-649, wherein a toxin activity of the second polypeptide is at least partly mitigated.

651. The method of any one of claims 641-650, wherein the first serotype is from a same bacteria as the second serotype.

652. The method of any one of claims 641-651, wherein the first serotype is from a different bacteria than the second serotype.

653. The method of any one of claims 641-652, wherein the first serotype is from Streptococcus pneumoniae.

654. The method of any one of claims 641-653, wherein the first serotype is from Neisseria meningitidis, Salmonella enterica, or Haemophilus influenzae.

655. The method of any one of claims 641-654, wherein the second serotype is from Streptococcus pneumoniae.

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656. The method of any one of claims 641-655, wherein the second bacteria is from Neisseria meningitidis, Salmonella enterica, or Haemophilus influenzae.

657. The method of any one of claims 641-656, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from Streptococcus pneumoniae, and the second serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F,24B, 31, 33F, 35F, 35B, or 38 from Streptococcus pneumoniae.

658. The method of any one of claims 641-657, wherein the first serotype comprises at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from Streptococcus pneumoniae, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from Streptococcus pneumoniae.

659. The method of any one of claims 641-658, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

660. The method of claim 659, wherein the adjuvant is an aluminum-cation comprising adjuvant.

661. The method of claim 660, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

662. The method of any one of claims 641-661, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

663. The method of any one of claims 641-662, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

664. The method of claim 663, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

665. The method of any one of claims 641-664, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

666. The method of any one of claims 641-665, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

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667. The method of any one of claims 641-666, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

668. The method of any one of claims 641-667, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

669. The method of any one of claims 641-668, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

670. The method of claim 669, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

671. The method of claim 670, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

672. The method claim 670, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

673. The method of any one of claims 641-672, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

674. The method of claim 673, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

675. The method of any one of claims 641-674, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

676. The method of any one of claims 641-675, wherein when the first immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

677. The method of any one of claims 641-676, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first

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immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

678. The method of any one of claims 641-677, wherein the first immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

679. The method of any one of claims 641-678, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

680. The method of claim 679, wherein the adjuvant is an aluminum-cation comprising adjuvant.

681. The method of claim 679, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

682. The method of any one of claims 641-681, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

683. The method of any one of claims 641-682, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

684. The method of claim 683, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

685. The method of any one of claims 641-684, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

686. The method of any one of claims 641-685, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

687. The method of claim 686, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

688. The method of any one of claims 641-687, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

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689. The method of any one of claims 641-688, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

690. The method of claim 689, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

691. The method of claim 690, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

692. The method of claim 690, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

693. The method of any one of claims 641-692, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

694. The method of claim 693, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

695. The method of any one of claims 641-694, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

696. The method of any one of claims 641-695, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

697. The method of any one of claims 641-696, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

698. The method of any one of claims 641-697, wherein the second immunogenic saccharide-polypeptide conjugate composition retains at least about 80% of its original biological

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activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

699. The method of any one of claims 641-698, wherein the first immunogenic saccharide-polypeptide conjugate composition is in unit dose form.

700. The method of any one of claims 641-699, wherein the second immunogenic saccharide-polypeptide conjugate composition is in unit dose form.

701. A method of making a composition comprising:

a) contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 566-584 with an excipient, an adjuvant, or any combination thereof.

702. The method of claim 701, wherein the composition is injectable or intranasally administrable.

703. The method claim 701, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

704. The method of any one of claims 701-703, wherein the composition is in the form of an intramuscularly injectable composition.

705. The method of any one of claims 701-704, further comprising contacting the composition with a chelating agent.

706. The method of claim 705, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

707. The method of any one of claims 701-706, wherein the composition is in a form of a suspension.

708. The method of any one of claims 701-707, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

709. The method of any one of claims 708, wherein the composition is in a form of a homogenous mixture.

710. The method of any one of claims 701-709, wherein the composition is contained in a pre-filled syringe.

711. The method of any one of claims 701-710, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

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712. The method of claim 711, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

713. The method of claim 712, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

714. The method of claim 713, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

715. The method of any one of claims 701-714, further comprising contacting the composition with a buffering agent.

716. The method of claim 715, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

717. The method of any one of claims 701-716, further comprising contacting the composition with sterile water.

718. The method of any one of claims 701-717, wherein when the composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

719. The method of any one of claims 701-718, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

720. The method of any one of claims 701-719, wherein the composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

721. A pharmaceutical composition comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a capsular polysaccharide serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A,

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11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38; each conjugated to a polypeptide.

722. The pharmaceutical composition of claim 721, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a capsular polysaccharide.

723. The pharmaceutical composition of claim 721, wherein the immunogenic saccharide-polypeptide conjugate(s) comprise a fragment of a capsular polysaccharide.

724. The pharmaceutical composition of claim 723, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

725. The pharmaceutical composition of any one of claims 721-724, wherein the polypeptide is an oligopeptide.

726. The pharmaceutical composition of any one of claims 721-725, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, or any fragment thereof.

727. The pharmaceutical composition of any one of claims 721-726, wherein a toxin activity of the polypeptide is at least partly mitigated.

728. The pharmaceutical composition of any one of claims 721-727, wherein the polypeptide comprises a mixture of polypeptides.

729. The pharmaceutical composition of claim 728, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein

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derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, any combination thereof.

730. The pharmaceutical composition of any one of claims 721-729, comprising at least 29 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35F, 35B, and 38.

731. The pharmaceutical composition of any one of claims 721-729, comprising at least 25 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.

732. The pharmaceutical composition of any one of claims 721-729, comprising at least 27 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24F, 33F, 35F, and 35B.

733. The pharmaceutical composition of any one of claims 721-729, comprising at least 19 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35B and 38.

734. A pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising a capsular polysaccharide serotype, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 20F, 22F, 23A, 23B, 24F, 31, 33F, 35B, and 38; each conjugated to a polypeptide.

735. The pharmaceutical composition of claim 734, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.

736. The pharmaceutical composition of claim 734, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

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