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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	P1143.70000US05
First Named Inventor	Bruce D. Forrest
Title	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Priority Mail Express® Label No.	VIA EFS WEB

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [X] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 117] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 15]
6. **Inventor's Oath or Declaration** [*Total Pages* 1] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [X] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [X] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [ ] **Other:**

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 23628 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Chelsea E. Witte-Garcia/	Date	June 14, 2021
Name (Print/Type)	Chelsea E. Witte-Garcia, Ph.D.	Registration No. (Attorney/Agent)	75,713

9321055.1

DECLARATION (37 C.F.R. § 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 C.F.R. § 1.76)

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

As the below named inventor, I declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number: PCT/US2017/025621

filed on March 31, 2017

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I have reviewed and understand the contents of the above-identified application, including the claims.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. § 1.56.

All statements made herein of my own knowledge are true, and all statements made herein on information and belief are believed to be true.

I hereby acknowledge that any willful false statement and the like made in this declaration is punishable by fine or imprisonment of not more than five (5) years, or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

LEGAL NAME OF SOLE OR FIRST INVENTOR

Inventor one: Bruce D. Forrest Date: Jan 15th, 2021

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor two: Jack D. Love Date: Jan 14, 2021

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor three: Date:

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor four: Date:

Signature:

☐ Additional inventors are being named on _____ Additional form(s) attached hereto.

Petitioner Merck Ex-1015, 0002

Application Data Sheet

Inventor Information

Inventor Number::	1
Given Name::	Bruce
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State or Province of mailing address::	FL
Country of mailing address::	US
Postal or Zip Code of mailing address::	33609

Correspondence Information

Correspondence Customer Number::	23628

Application Information

Application Type::	Nonprovisional
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Attorney Docket Number::	P1143.70000US05
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	15
Small Entity?::	Yes
Petition included?::	No

Petitioner Merck Ex-1015, 0004

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No

Representative Information

Representative Customer Number::	23628

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Prior Appl Status::
This Application	Continuation of	16/089040	09/27/18	Pending
16/089040	a 371 of international	PCT/US2017/025621	03/31/17	
PCT/US2017/025621	Claims benefit of provisional	62/373807	08/11/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/372263	08/08/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/330245	05/02/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/316555	03/31/16	Expired

Applicant Information

Applicant Number::	1
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City of mailing address::	South Nyack

State or Province of mailing address::	NY
Country of mailing address::	US
Postal or Zip Code of mailing address::	10960

Assignee Information Including Non-Applicant Assignee Information

Assignee Number::	1
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Street of mailing address::	135 Piermont Avenue
City of mailing address::	South Nyack
State or Province of mailing address::	NY
Country of mailing address::	US
Postal or Zip Code of mailing address::	10960

Petitioner Merck Ex-1015, 0006

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.

Petitioner Merck Ex-1015, 0007

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	/Chelsea E. Witte-Garcia/	Date (YYYY-MM-DD)	2021-06-14
Name	Chelsea E. Witte-Garcia, Ph.D.	Registration Number	75,713

Petitioner Merck Ex-1015, 0008

Electronic Acknowledgement Receipt

EFS ID:	42983687
Application Number:	17347435
International Application Number:	
Confirmation Number:	4020
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. Forrest
Customer Number:	23628
Filer:	Chelsea E. Witte-Garcia/Patricia McDonald
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US05
Receipt Date:	14-JUN-2021
Filing Date:	
Time Stamp:	20:27:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		P114370000US05-PAMN-CEW.pdf	24128 0b187ea1cf208e9d6c46875f8017c61172d9bd98	yes	3

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Preliminary Amendment	1	1
	Specification	2	2
	Applicant Arguments/Remarks Made in an Amendment	3	3

Warnings:					
Information:					
2		P114370000US05-UTLAPL-CEW.pdf	349114 e050015d60e5381af0c2f3565e256913aa524d89	yes	117

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	87
	Claims	88	116
	Abstract	117	117

Warnings:					
Information:					
3	Drawings-other than black and white line drawings	P114370000US05-DRW-CEW.pdf	1197986 729b191a7ac6db263b28877ca36bec1fe7fa176d	no	15
Warnings:					
Information:					
4	Transmittal of New Application	P114370000US05-TRN-CEW.pdf	33089 081f5411e9e92e1a29b0bc180e81ab9377fa2a50	no	1
Warnings:					
Information:					
5	Oath or Declaration filed	P114370000US05-INVDEC-CEW.pdf	167311 27c632dce6d19b3b91d4da4331636374c16d842e	no	1
Warnings:					
Information:					

6	Application Data Sheet	P114370000US05-ADS-CEW.pdf	63961 27548d606cb265efa5ea761c8e222cc90512c276	no	6

Warnings:	
Information:	
This is not an USPTO supplied ADS fillable form	
Total Files Size (in bytes):	1835589

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.

Docket No.: P1143.70000US05
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	Not Yet Assigned
Confirmation No.:	Not Yet Assigned
Filed:	Concurrently Herewith
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned

PRELIMINARY AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Prior to examination on the merits, please amend the above-referenced U.S. Patent Application as follows:

Amendments to the Specification begin on page 2 of this paper.

Remarks begin on page 3 of this paper.

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AMENDMENTS TO THE SPECIFICATION

Please replace paragraph **[0001]** with the following amended paragraph:

[0001] This application is a continuation of and claims under 35 U.S.C. § 120 to U.S. Patent Application U.S.S.N. 16/089,040, filed September 27, 2018, which is a national stage filing under 35 U.S.C. § 371 of International PCT Application PCT/US2017/025621, filed March 31, 2017, which ~~is related to~~ claims priority under 35 U.S.C. § 119(e) to U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/316,555, filed on March 31, 2016; U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/330,245, filed on May 2, 2016; U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/372,263, filed on August 8, 2016; and U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/373,807, filed on August 11, 2016, each of which [[are]] is herein incorporated by reference ~~in their entirety~~.

REMARKS

Applicant requests entrance of this Preliminary Amendment to update the "RELATED APPLICATIONS" section of this Application. Applicant reserves the right to pursue subject matter canceled from the claims in future applications claiming priority to the present Application. Applicant submits that no new matter is added to the Application by this Preliminary Amendment.

Dated: June 14, 2021

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
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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.

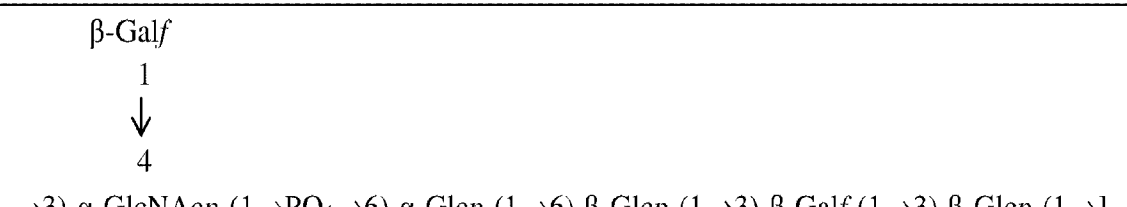
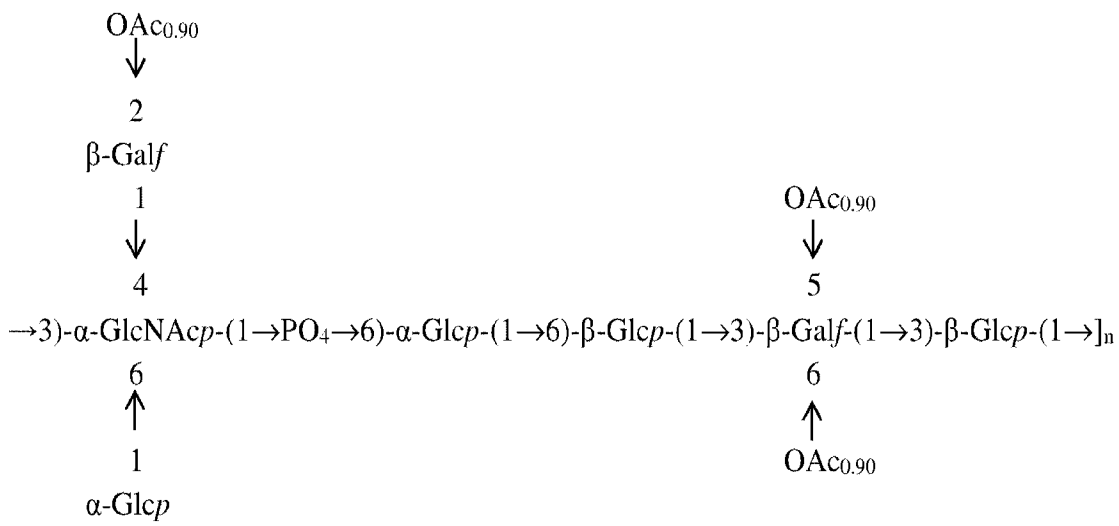
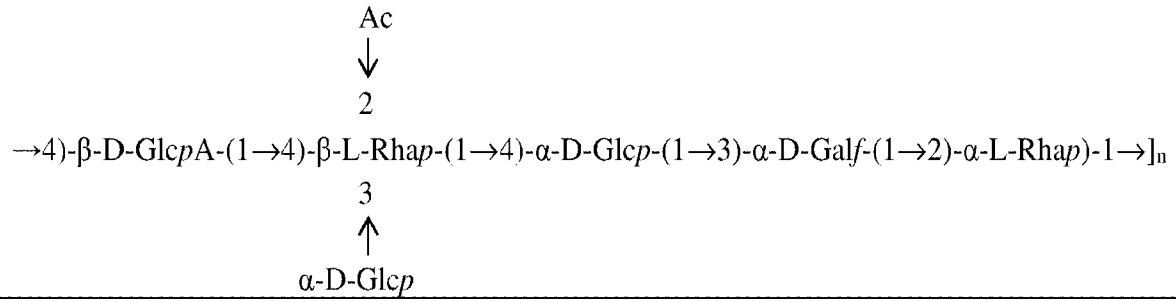
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 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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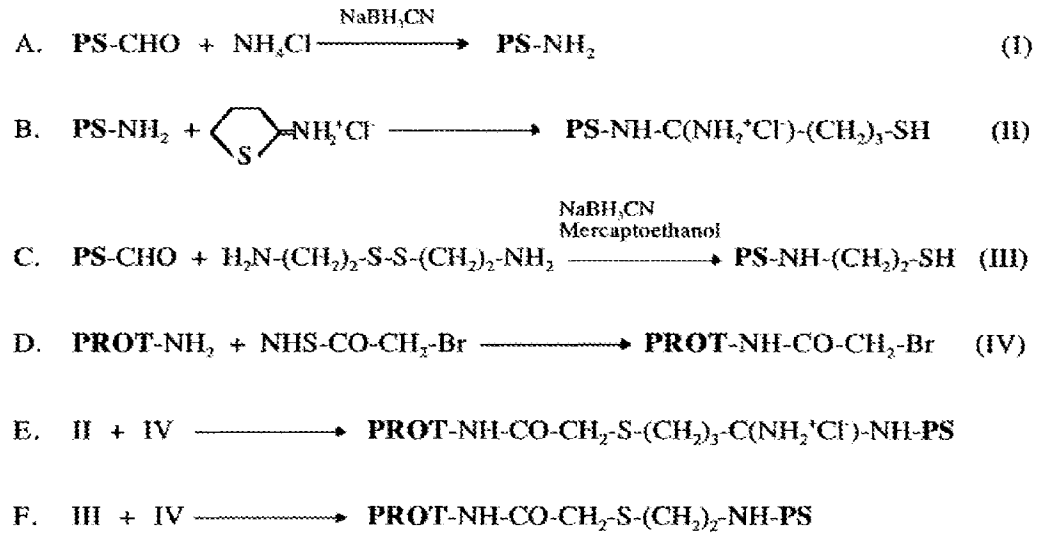
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[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.*, Oxydcoxynucleotides 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 Palmitate, 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.

Petitioner Merck Ex-1015, 0130

ABSTRACT OF THE DISCLOSURE

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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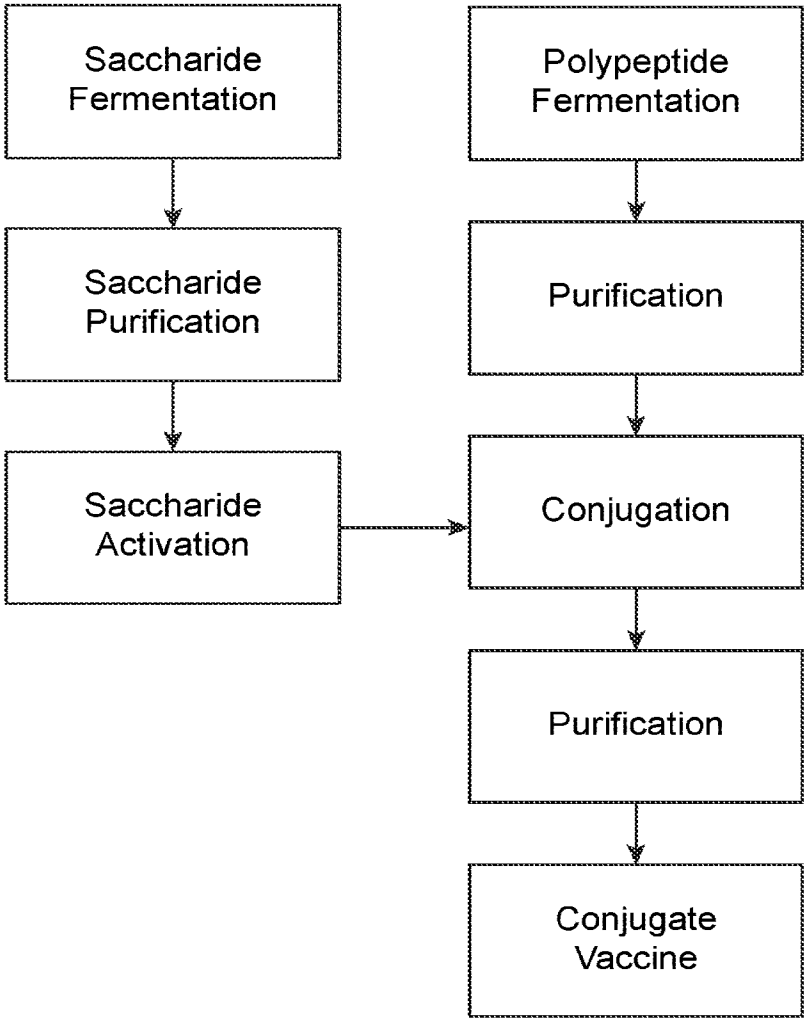

Saccharide Fermentation
Saccharide Purification
Saccharide Activation
Polypeptide Fermentation
Purification
Conjugation
Purification
Conjugate Vaccine

FIG. 1

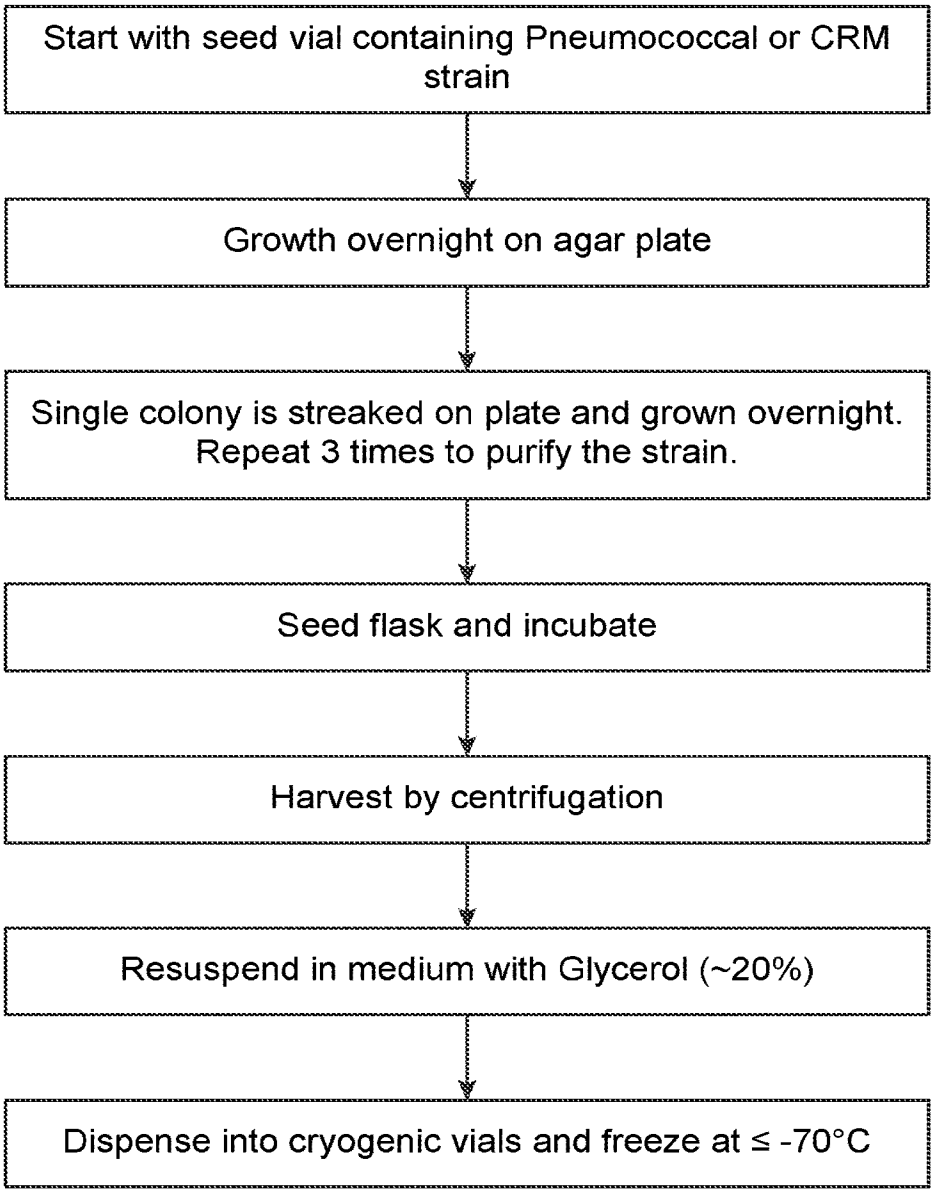

Start with seed vial containing Pneumococcal or CRM strain
Growth overnight on agar plate
Single colony is streaked on plate and grown overnight. Repeat 3 times to purify the strain.
Seed flask and incubate
Harvest by centrifugation
Resuspend in medium with Glycerol (~20%)
Dispense into cryogenic vials and freeze at ≤ -70°C

FIG. 2

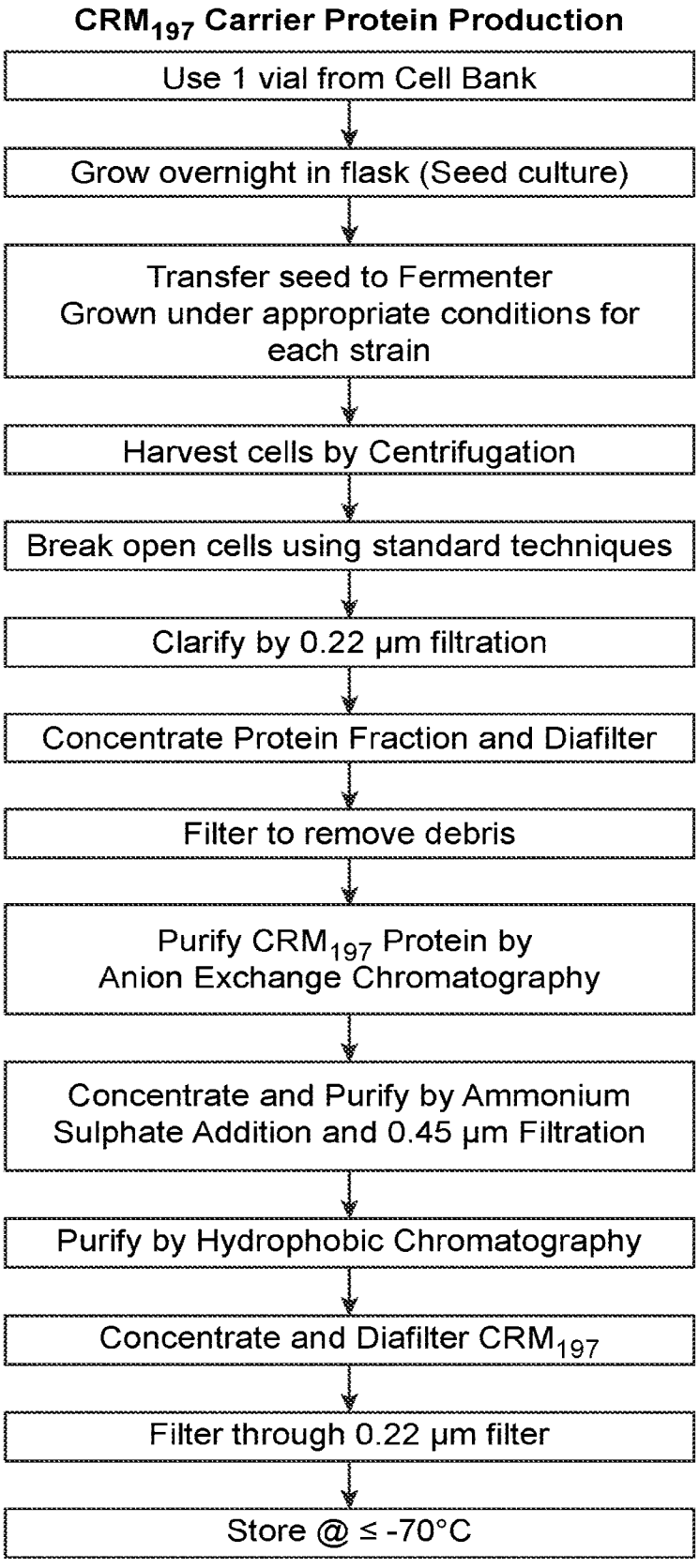

CRM197 Carrier Protein Production
Use 1 vial from Cell Bank
Grow overnight in flask (Seed culture)
Transfer seed to Fermenter
Grown under appropriate conditions for each strain
Harvest cells by Centrifugation
Break open cells using standard techniques
Clarify by 0.22 µm filtration
Concentrate Protein Fraction and Diafilter
Filter to remove debris
Purify CRM197 Protein by Anion Exchange Chromatography
Concentrate and Purify by Ammonium Sulphate Addition and 0.45 µm Filtration
Purify by Hydrophobic Chromatography
Concentrate and Diafilter CRM197
Filter through 0.22 µm filter
Store @ ≤ -70°C

FIG. 3

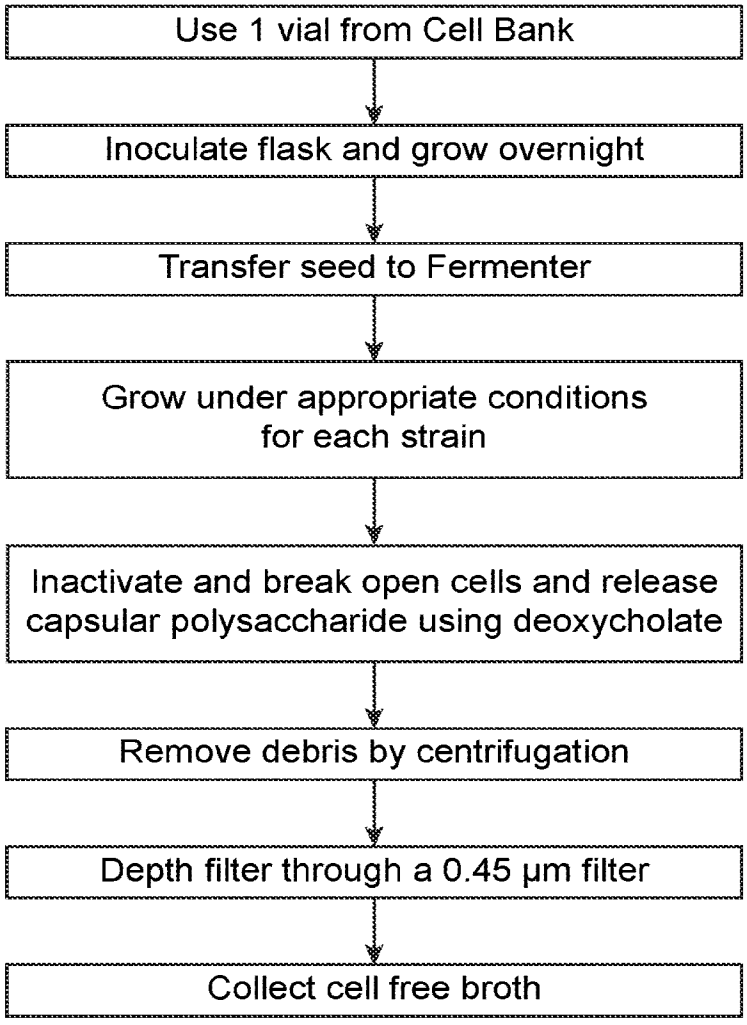

Use 1 vial from Cell Bank
Inoculate flask and grow overnight
Transfer seed to Fermenter
Grow under appropriate conditions for each strain
Inactivate and break open cells and release capsular polysaccharide using deoxycholate
Remove debris by centrifugation
Depth filter through a 0.45 µm filter
Collect cell free broth

FIG. 4

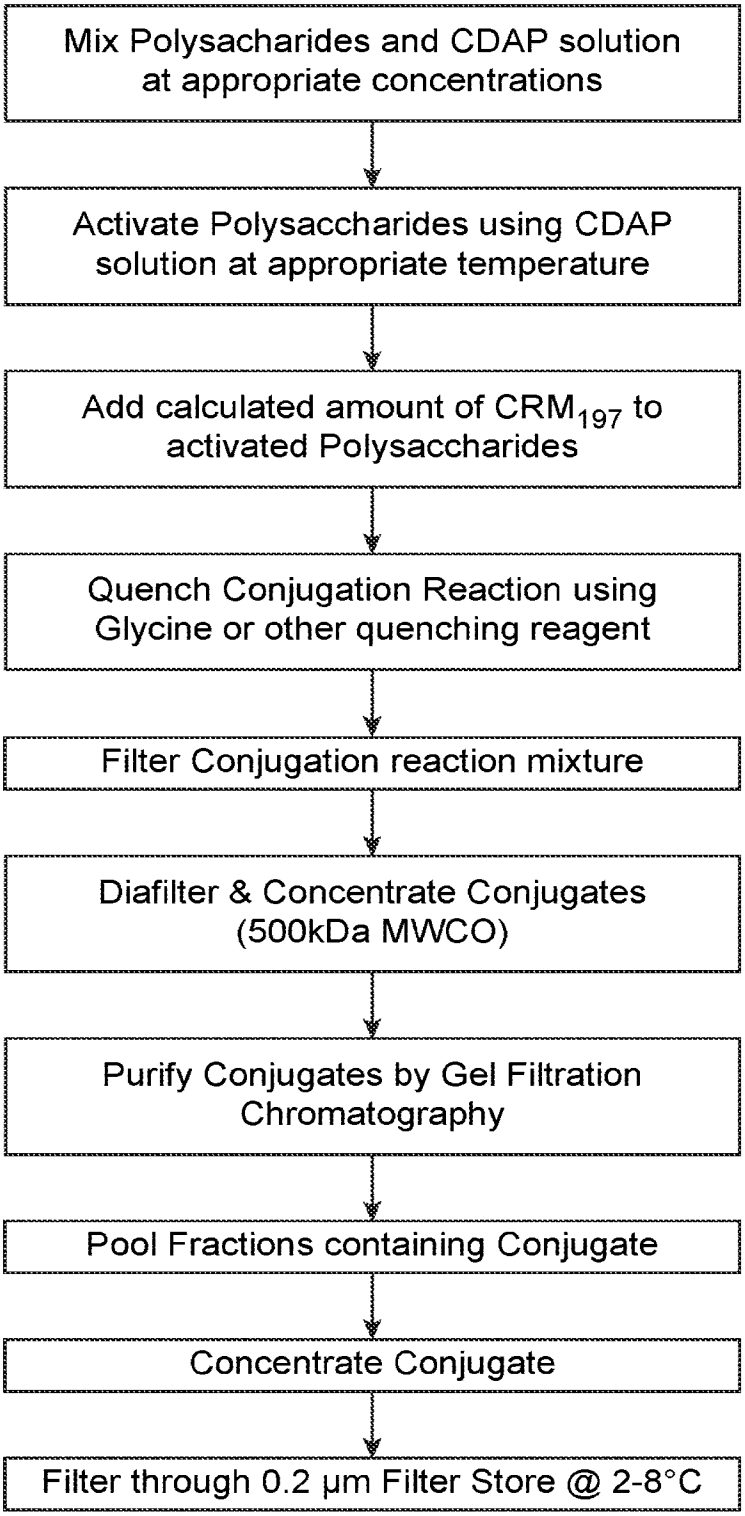

Mix Polysacharides and CDAP solution at appropriate concentrations
Activate Polysaccharides using CDAP solution at appropriate temperature
Add calculated amount of CRM_{197} to activated Polysaccharides
Quench Conjugation Reaction using Glycine or other quenching reagent
Filter Conjugation reaction mixture
Diafilter & Concentrate Conjugates (500kDa MWCO)
Purify Conjugates by Gel Filtration Chromatography
Pool Fractions containing Conjugate
Concentrate Conjugate
Filter through 0.2 µm Filter Store @ 2-8°C

FIG. 5

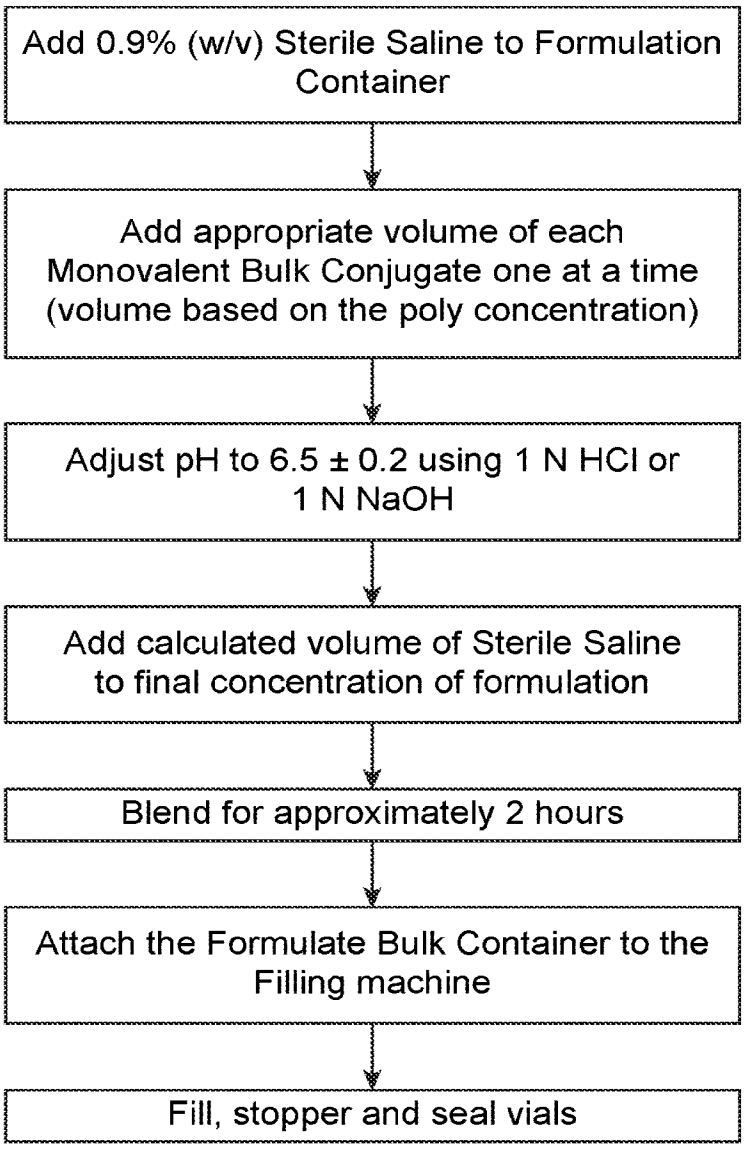

Add 0.9% (w/v) Sterile Saline to Formulation Container
Add appropriate volume of each Monovalent Bulk Conjugate one at a time (volume based on the poly concentration)
Adjust pH to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH
Add calculated volume of Sterile Saline to final concentration of formulation
Blend for approximately 2 hours
Attach the Formulate Bulk Container to the Filling machine
Fill, stopper and seal vials

FIG. 6

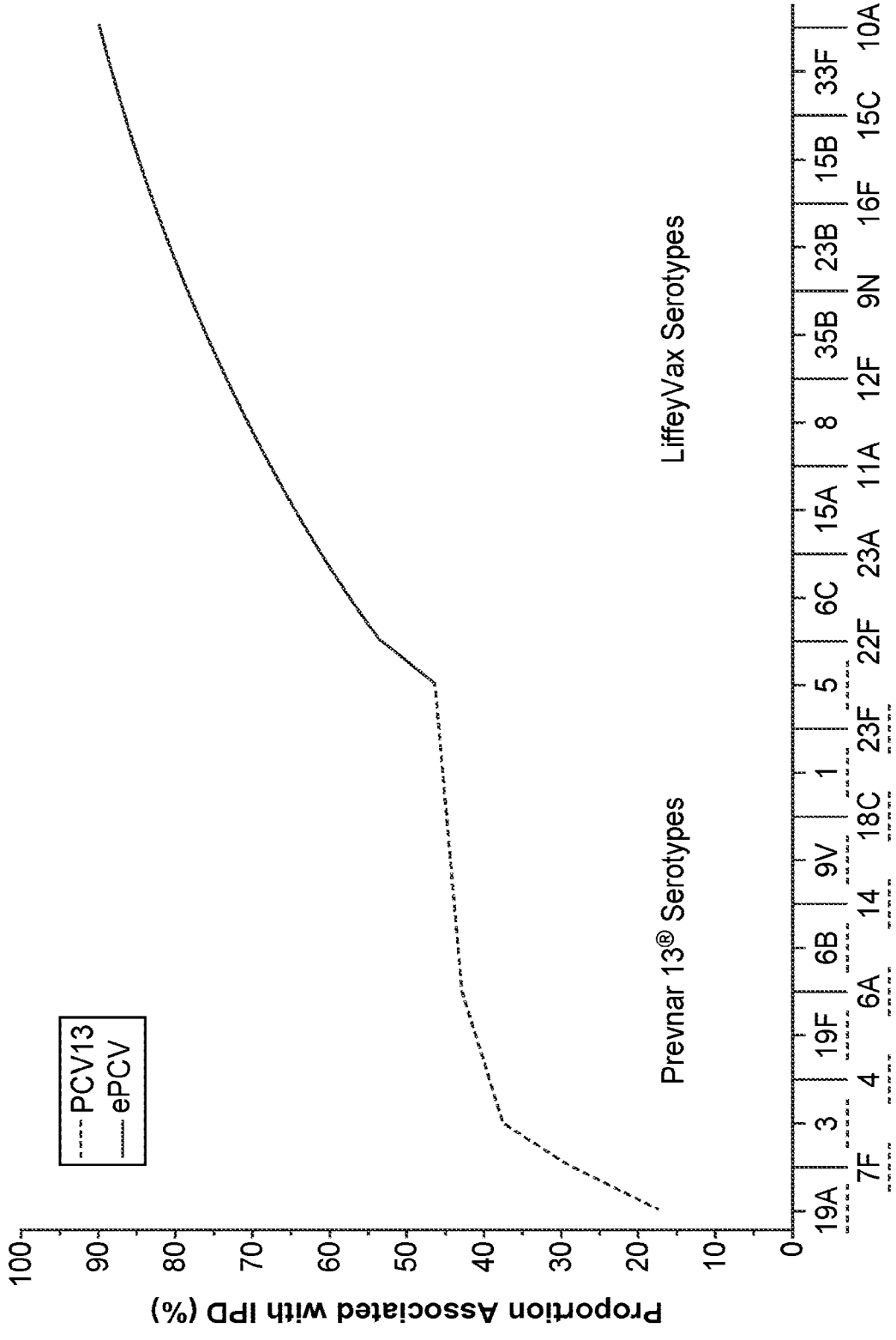

PCV13
ePCV
Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13® Serotypes
LiffeyVax Serotypes
19A
7F
3
4
19F
6A
6B
14
9V
18C
1
23F
5
22F
6C
23A
15A
11A
8
12F
35B
9N
23B
16F
15B
15C
33F
10A
S. pneumoniae Serotypes

FIG. 7

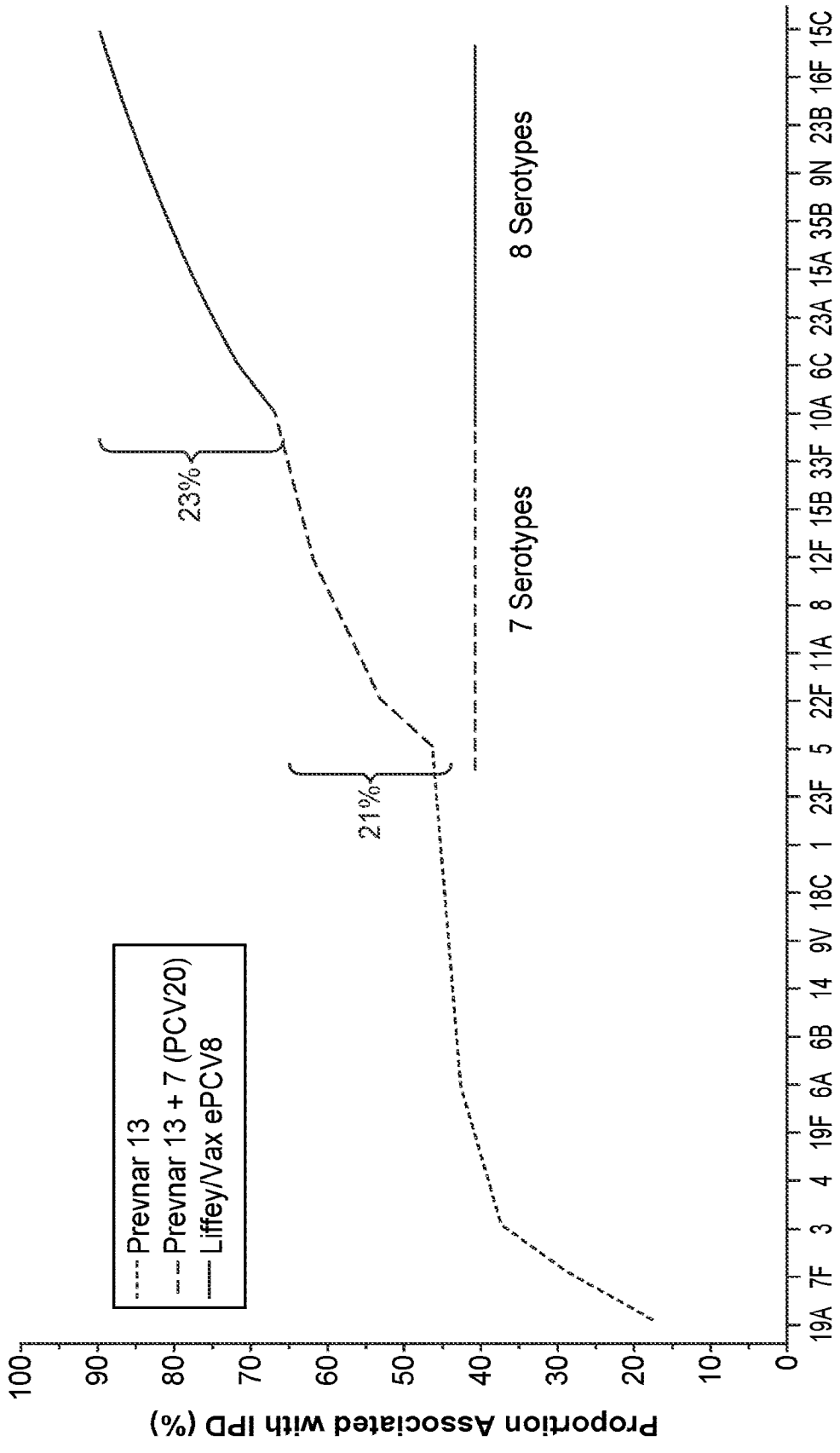

Prevnar 13
Prevnar 13 + 7 (PCV20)
Liffey/Vax ePCV8
23%
21%
7 Serotypes
8 Serotypes
Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
19A 7F 3 4 19F 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C
S. pneumoniae Serotypes

FIG. 8

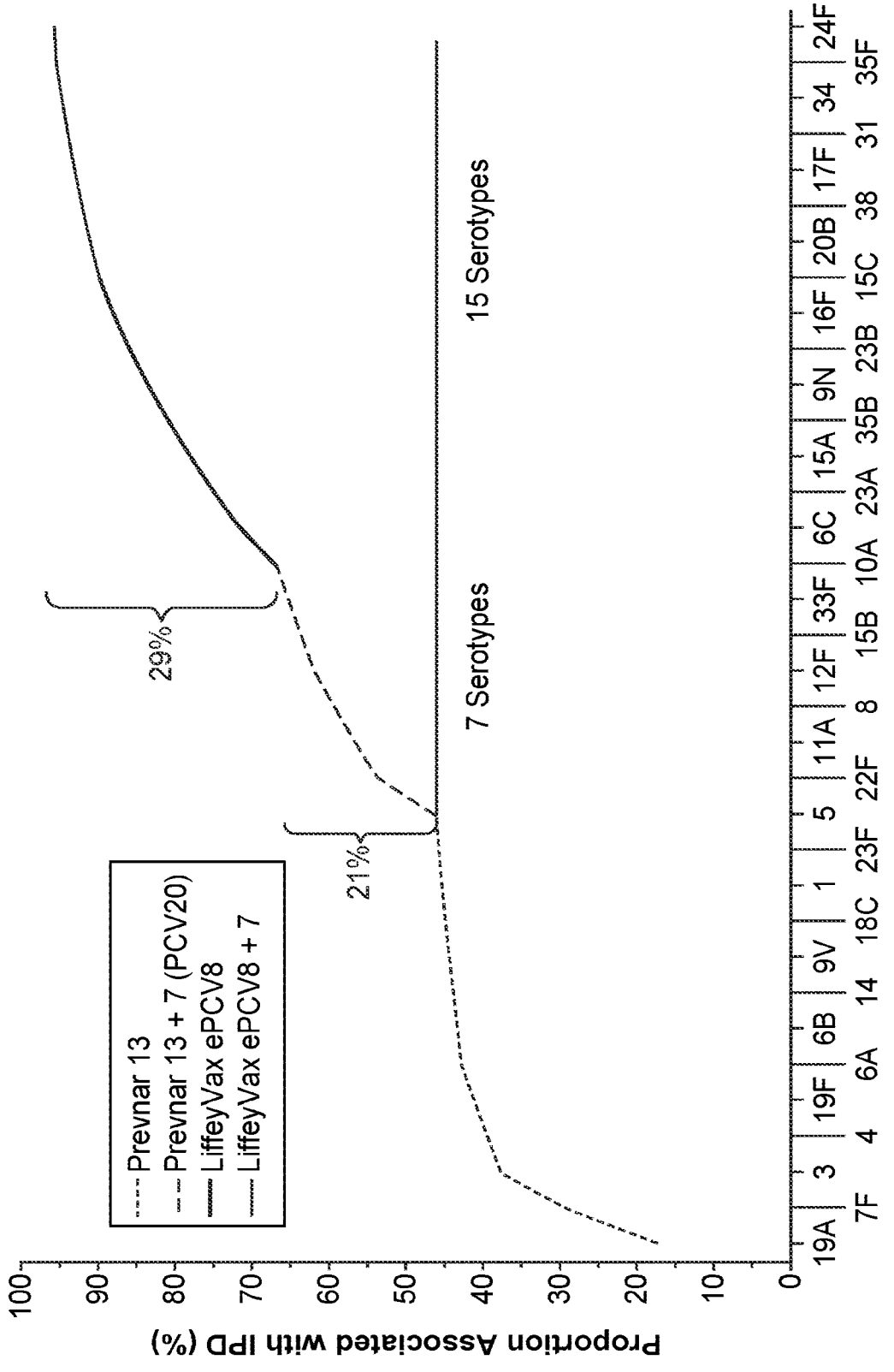

Prevnar 13
Prevnar 13 + 7 (PCV20)
LiffeyVax ePCV8
LiffeyVax ePCV8 + 7
29%
21%
7 Serotypes
15 Serotypes
Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
19A 3 7F 19F 4 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C 20B 38 17F 31 34 35F 24F
S. pneumoniae Serotypes

FIG. 9

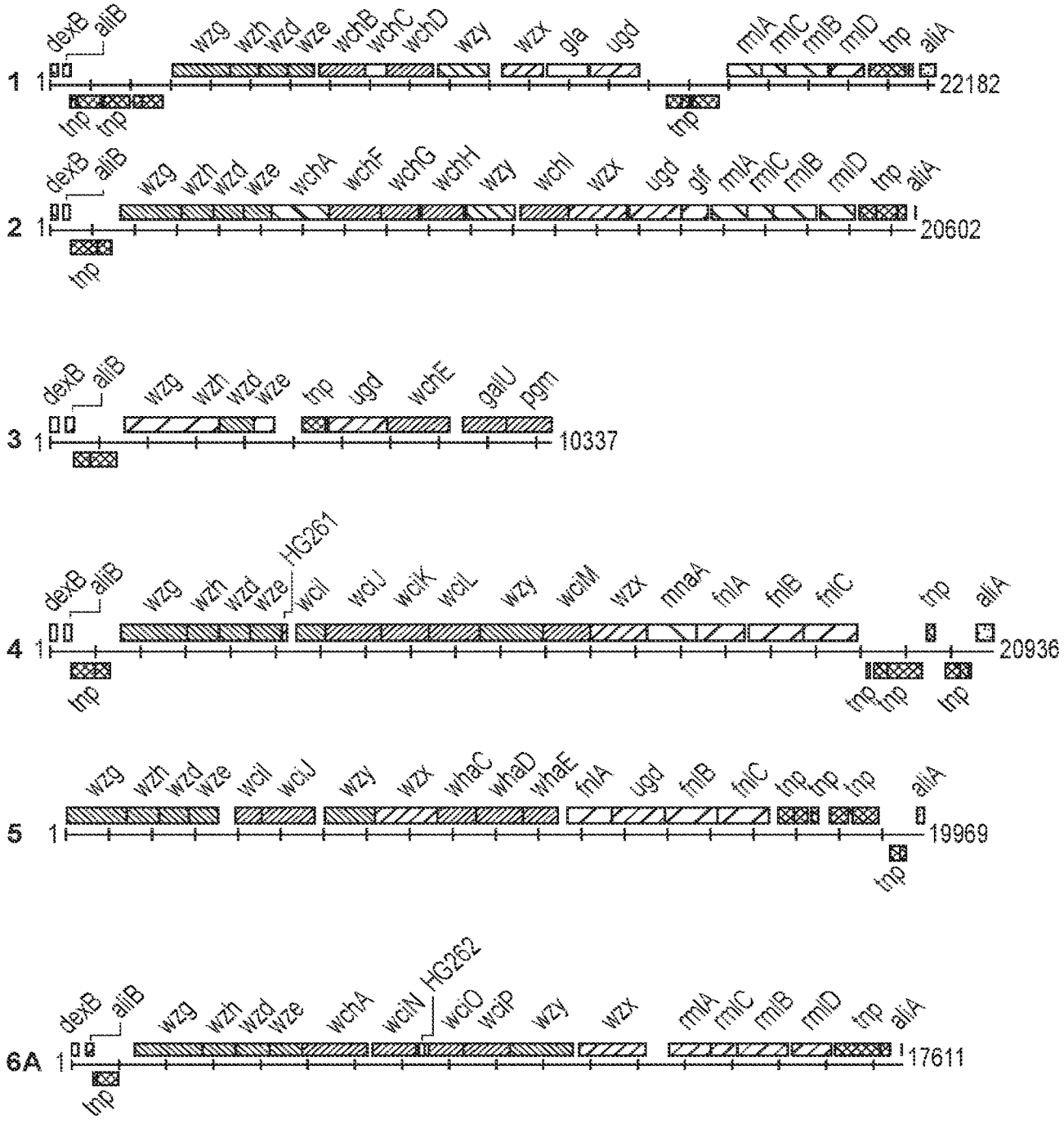

1
dexB
aliB
wzg
wzh
wzd
wze
wchB
wchC
wchD
wzy
wzx
gla
ugd
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
22182
tnp
tnp
tnp
2
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wchG
wchH
wzy
wchI
wzx
ugd
glf
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
20602
tnp
3
dexB
aliB
wzg
wzh
wzd
wze
tnp
ugd
wchE
galU
pgm
1
10337
4
dexB
aliB
wzg
wzh
wzd
wze
HG261
wcil
wciJ
wciK
wciL
wzy
wciM
wzx
nnaA
fnlA
fnlB
fnlC
tnp
aliA
1
20936
tnp
tnp
tnp
tnp
5
wzg
wzh
wzd
wze
wcil
wciJ
wzy
wzx
whaC
whaD
whaE
fnlA
ugd
fnlB
fnlC
tnp
tnp
tnp
aliA
1
19969
tnp
6A
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciN
HG262
wciO
wciP
wzy
wzx
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
17611
tnp

FIG. 10A

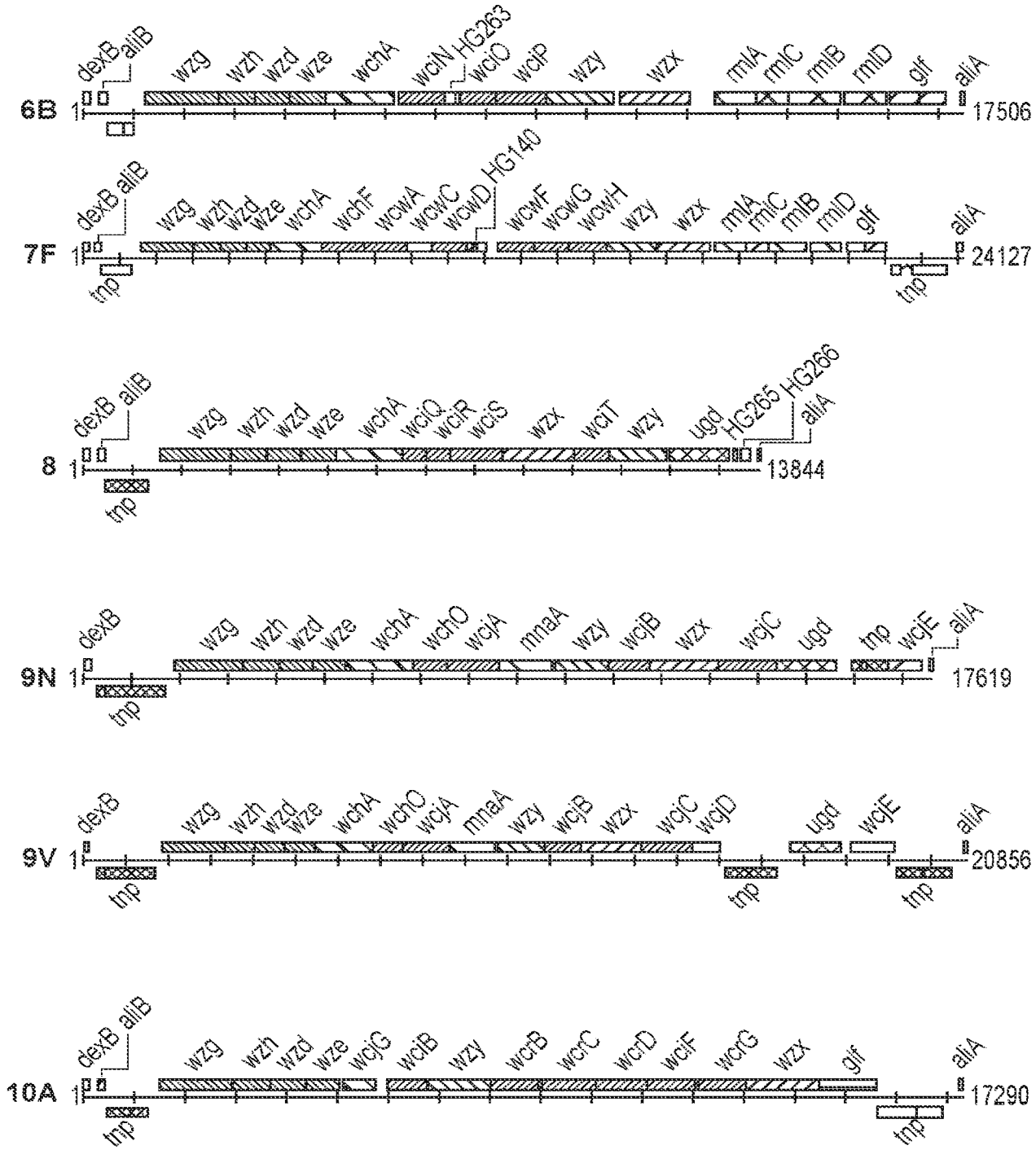

6B
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciN
HG263
wciO
wciP
wzy
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
1
17506
7F
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wcwA
wcwC
wcwD
HG140
wcwF
wcwG
wcwH
wzy
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
tnp
tnp
1
24127
8
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciQ
wciR
wciS
wzx
wciT
wzy
ugd
HG265
HG266
aliA
tnp
1
13844
9N
dexB
wzg
wzh
wzd
wze
wchA
wchO
wcjA
mnaA
wzy
wcjB
wzx
wcjC
ugd
tnp
wcjE
aliA
tnp
1
17619
9V
dexB
wzg
wzh
wzd
wze
wchA
wchO
wcjA
mnaA
wzy
wcjB
wzx
wcjC
wcjD
ugd
wcjE
aliA
tnp
tnp
tnp
1
20856
10A
dexB
aliB
wzg
wzh
wzd
wze
wcjG
wciB
wzy
wciB
wciC
wciD
wciF
wciG
wzx
glf
aliA
tnp
tnp
1
17290

FIG. 10B

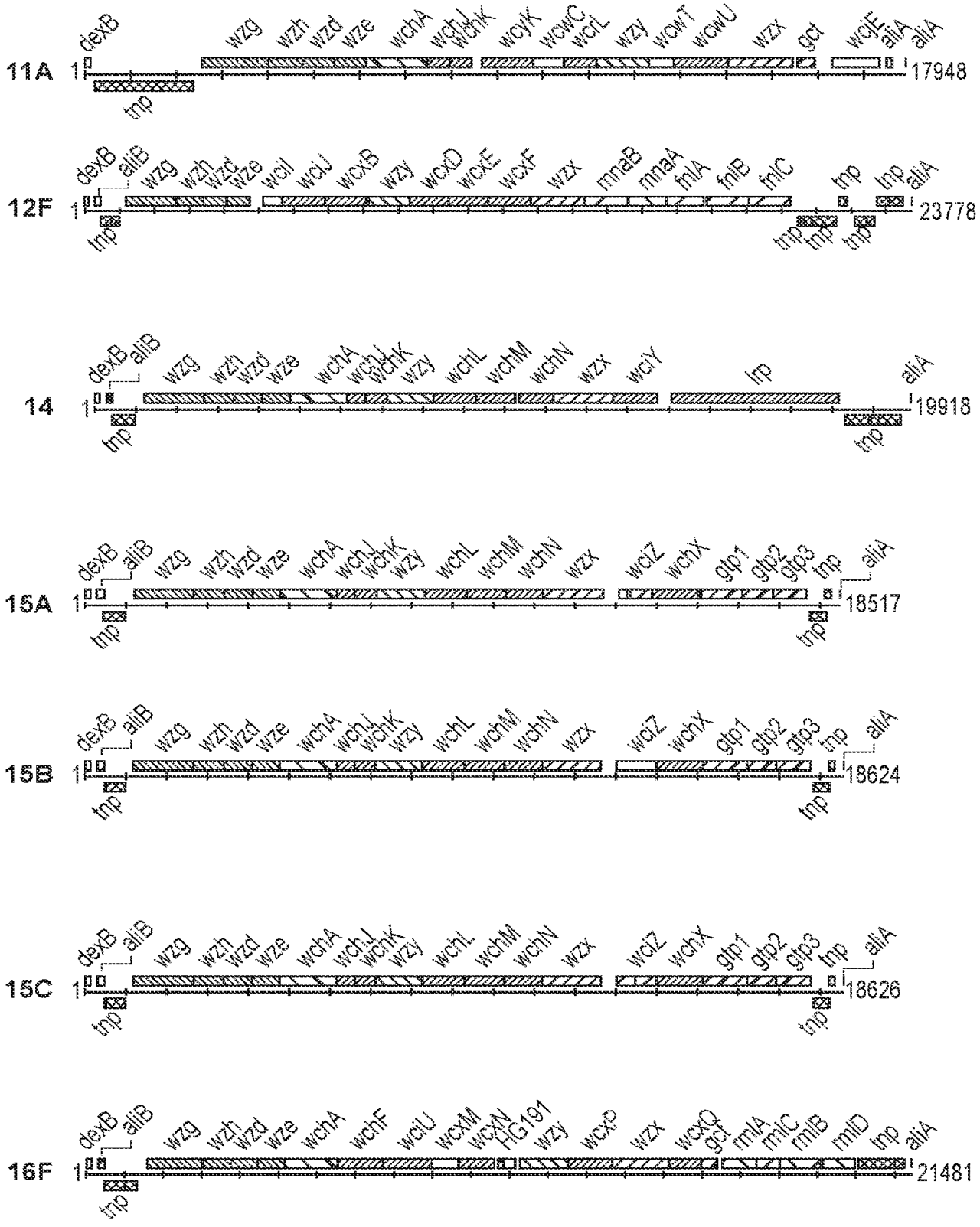

11A
dexB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wcyK
wcwC
wcrL
wzy
wcwT
wcwU
wzx
gct
wcjE
aliA
aliA
tnp
1
17948
12F
dexB
aliB
wzg
wzh
wzd
wze
wciI
wciJ
wcxB
wzy
wcxD
wcxE
wcxF
wzx
rmaB
rmaA
fnlA
fnlB
fnlC
tnp
tnp
aliA
tnp
tnp
tnp
tnp
1
23778
14
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciY
trp
aliA
tnp
tnp
1
19918
15A
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
glp1
glp2
glp3
tnp
aliA
tnp
tnp
1
18517
15B
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
glp1
glp2
glp3
tnp
aliA
tnp
tnp
1
18624
15C
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
glp1
glp2
glp3
tnp
aliA
tnp
tnp
1
18626
16F
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wciU
wcxM
wcxN
HG191
wzy
wcxP
wzx
wcxQ
gct
rmlA
rmlC
rmlB
rmlD
tnp
aliA
tnp
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21481

FIG. 10C

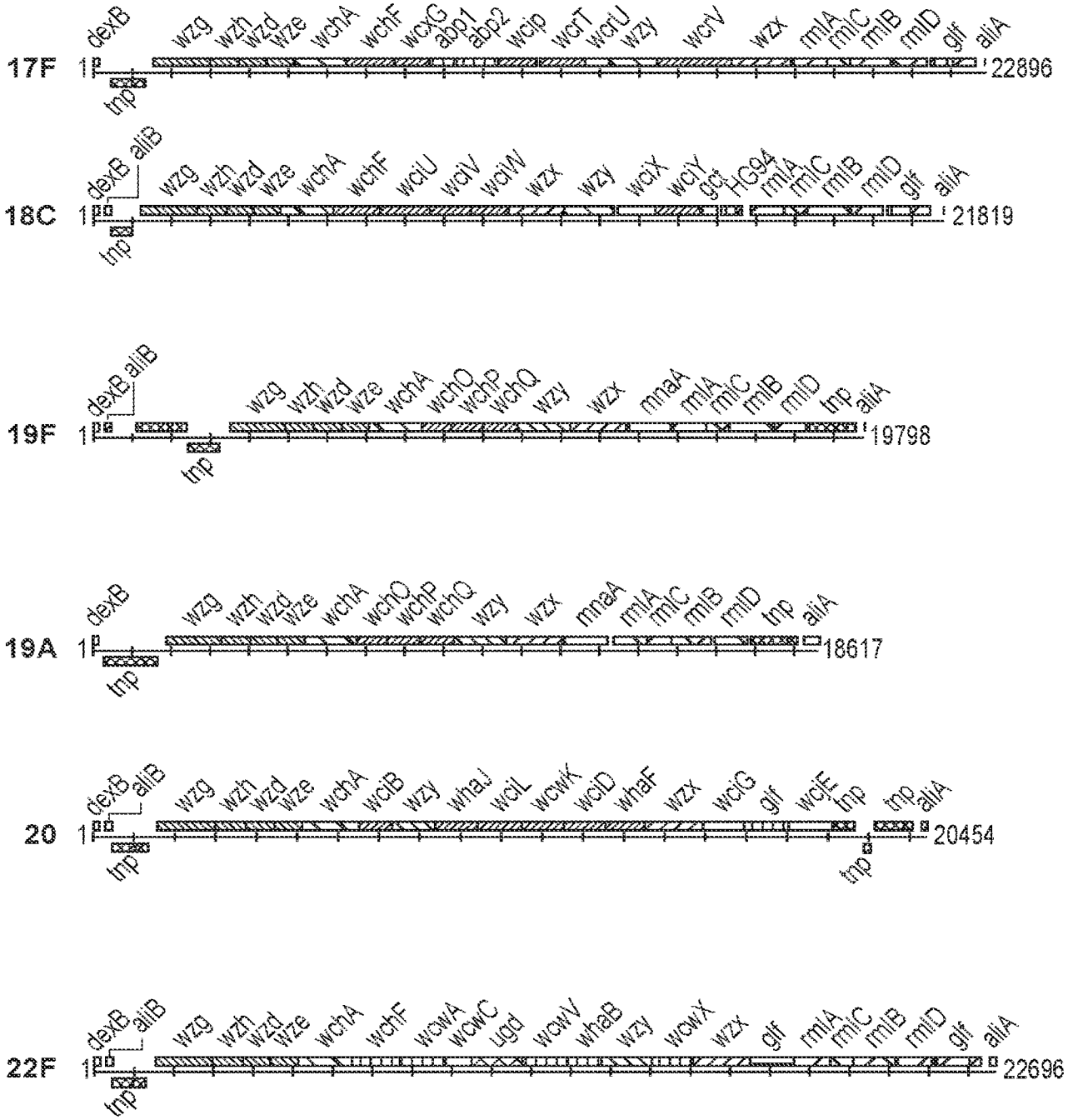

17F
dexB
wzg wzh wzd wze wchA wchF wcxG abp1 abp2 wcip wcrT wcrU wzy wcrV wzx rmlA rmlC rmlB rmlD glf aliA
1
22896
tnp
18C
dexB aliB
wzg wzh wzd wze wchA wchF wciU wciV wciW wzx wzy wciX wciY gct HG9A rmlA rmlC rmlB rmlD glf aliA
1
21819
tnp
19F
dexB aliB
wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx mnaA rmlA rmlC rmlB rmlD tnp aliA
1
19798
tnp
19A
dexB
wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx mnaA rmlA rmlC rmlB rmlD tnp aliA
1
18617
tnp
20
dexB aliB
wzg wzh wzd wze wchA wciB wzy whaJ wciL wcwK wciD whaF wzx wciG glf wciE tnp tnp aliA
1
20454
tnp
tnp
22F
dexB aliB
wzg wzh wzd wze wchA wchF wcwA wcwC ugd wcwV whaB wzy wcwX wzx glf rmlA rmlC rmlB rmlD glf aliA
1
22696
tnp

FIG. 10D

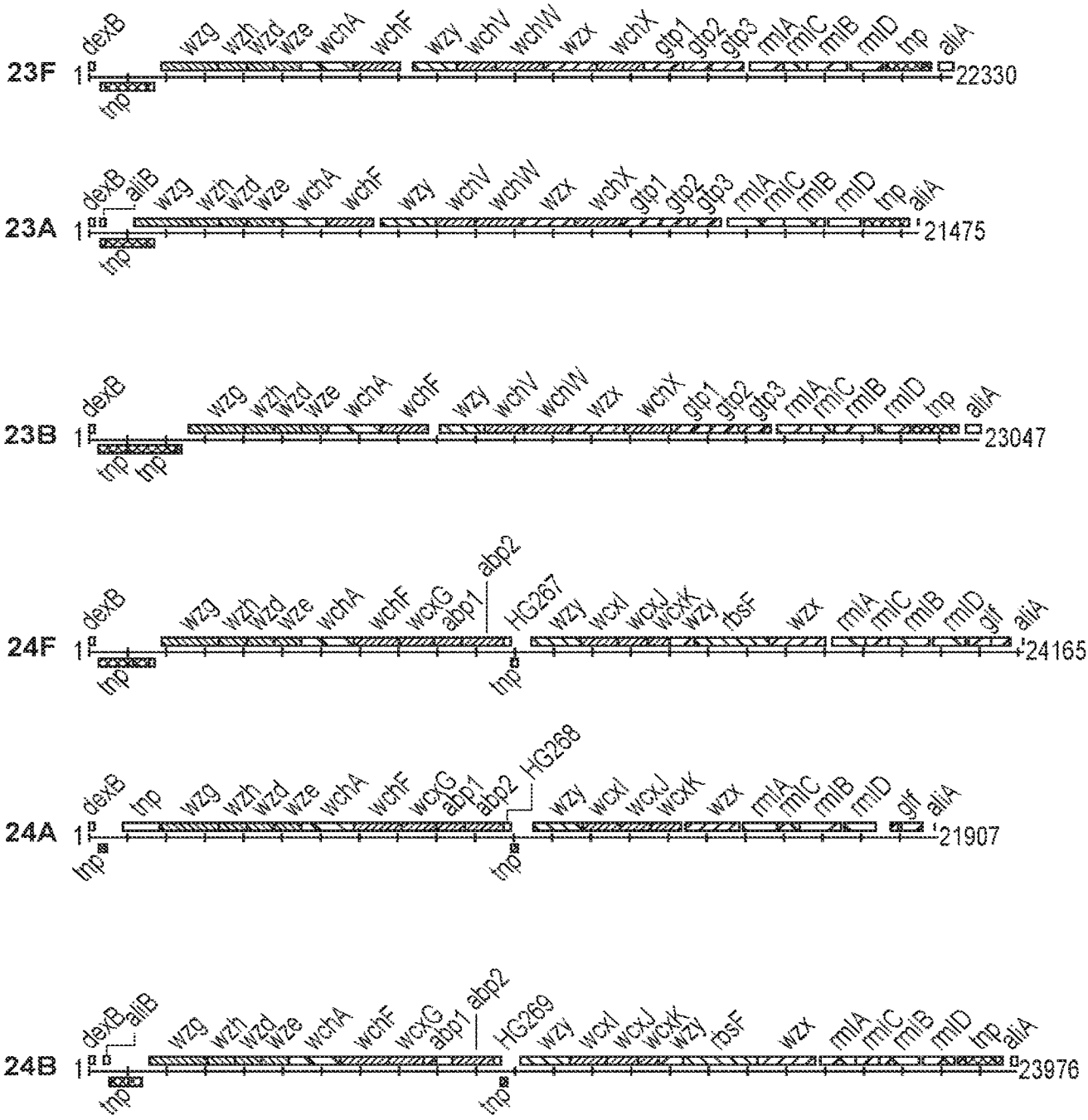

23F
dexB
wzg wzh wzd wze wchA wchF wzy wchV wchW wzx wchX glp1 glp2 glp3 rmlA rmlC rmlB rmlD tnp aliA
1
22330
tnp
23A
dexB aliB
wzg wzh wzd wze wchA wchF wzy wchV wchW wzx wchX glp1 glp2 glp3 rmlA rmlC rmlB rmlD tnp aliA
1
21475
tnp
23B
dexB
wzg wzh wzd wze wchA wchF wzy wchV wchW wzx wchX glp1 glp2 glp3 rmlA rmlC rmlB rmlD tnp aliA
1
23047
tnp tnp
24F
dexB
wzg wzh wzd wze wchA wchF wcxG abp1 abp2 HG267 wzy wcxI wcxJ wcxK wzy rbsF wzx rmlA rmlC rmlB rmlD glf aliA
1
24165
tnp tnp
24A
dexB tnp
wzg wzh wzd wze wchA wchF wcxG abp1 abp2 HG268 wzy wcxI wcxJ wcxK wzx rmlA rmlC rmlB rmlD glf aliA
1
21907
tnp tnp
24B
dexB aliB
wzg wzh wzd wze wchA wchF wcxG abp1 abp2 HG269 wzy wcxI wcxJ wcxK wzy rbsF wzx rmlA rmlC rmlB rmlD tnp aliA
1
23976
tnp tnp

FIG. 10E

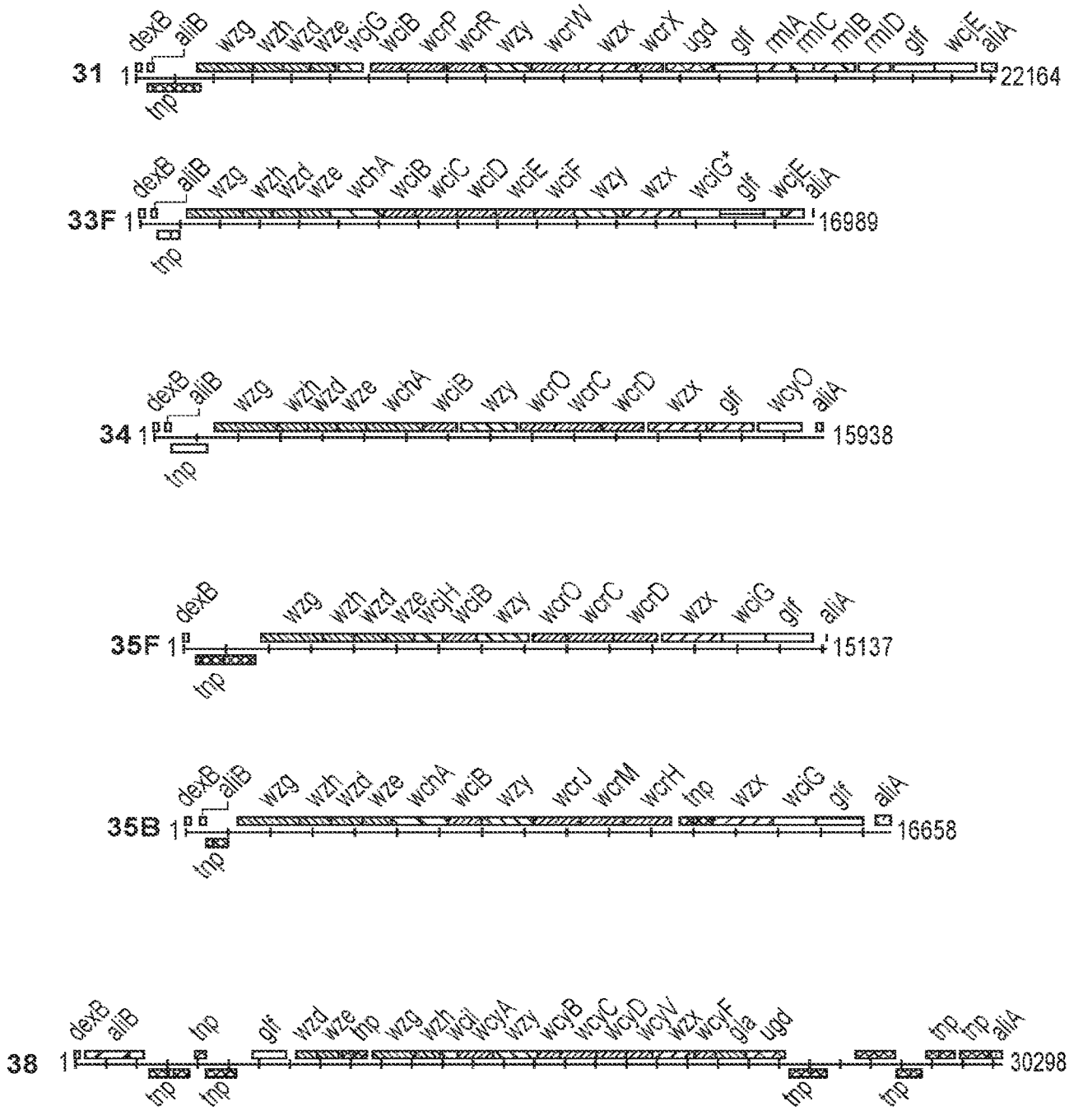

31
dexB
aliB
wzg
wzh
wzd
wze
wciG
wciB
wcrP
wcrR
wzy
wcrW
wzx
wcrX
ugd
glf
rmlA
rmlC
rmlB
rmlD
glf
wciE
aliA
tnp
1
22164
33F
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wciC
wciD
wciE
wciF
wzy
wzx
wciG*
glf
wciE
aliA
tnp
1
16989
34
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wzy
wcrO
wcrC
wcrD
wzx
glf
wcyO
aliA
tnp
1
15938
35F
dexB
wzg
wzh
wzd
wze
wciH
wciB
wzy
wcrO
wcrC
wcrD
wzx
wciG
glf
aliA
tnp
1
15137
35B
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wzy
wcrJ
wcrM
wcrH
tnp
wzx
wciG
glf
aliA
tnp
1
16658
38
dexB
aliB
tnp
glf
wzd
wze
tnp
wzg
wzh
wciI
wcyA
wzy
wcyB
wcyC
wcyD
wcyV
wzx
wcyF
gla
ugd
tnp
tnp
tnp
aliA
tnp
tnp
1
30298

FIG. 10F

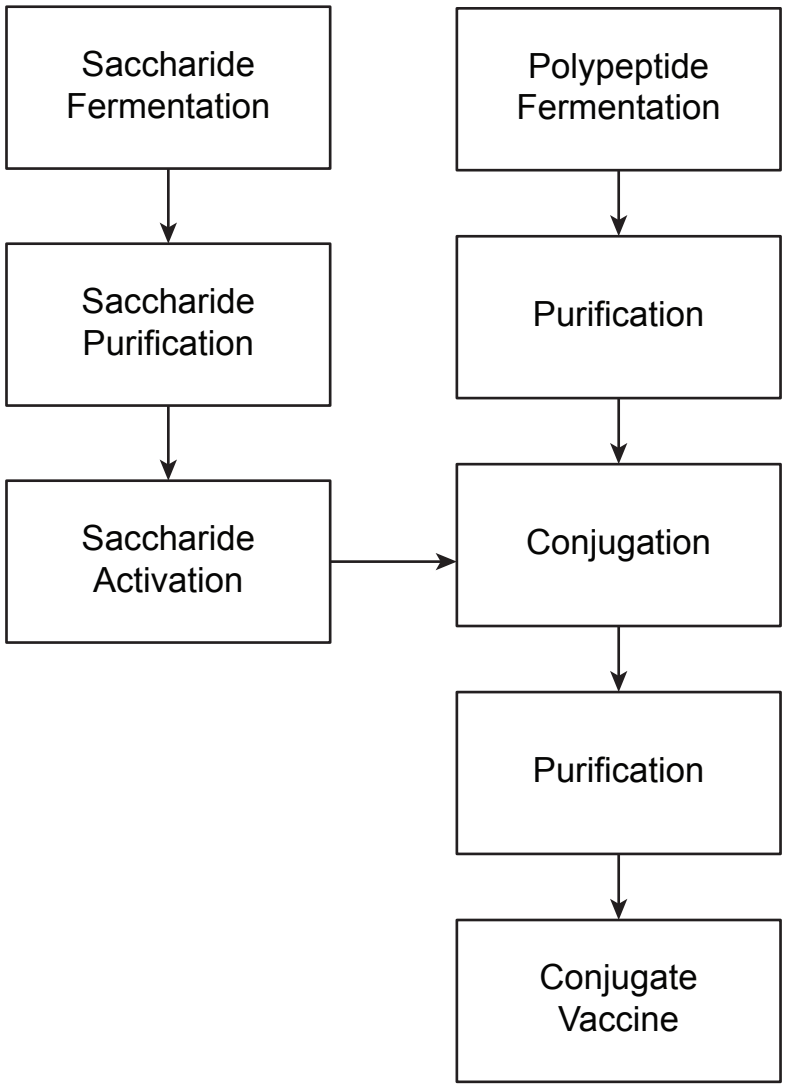

Saccharide Fermentation
Polypeptide Fermentation
Saccharide Purification
Purification
Saccharide Activation
Conjugation
Purification
Conjugate Vaccine

FIG. 1

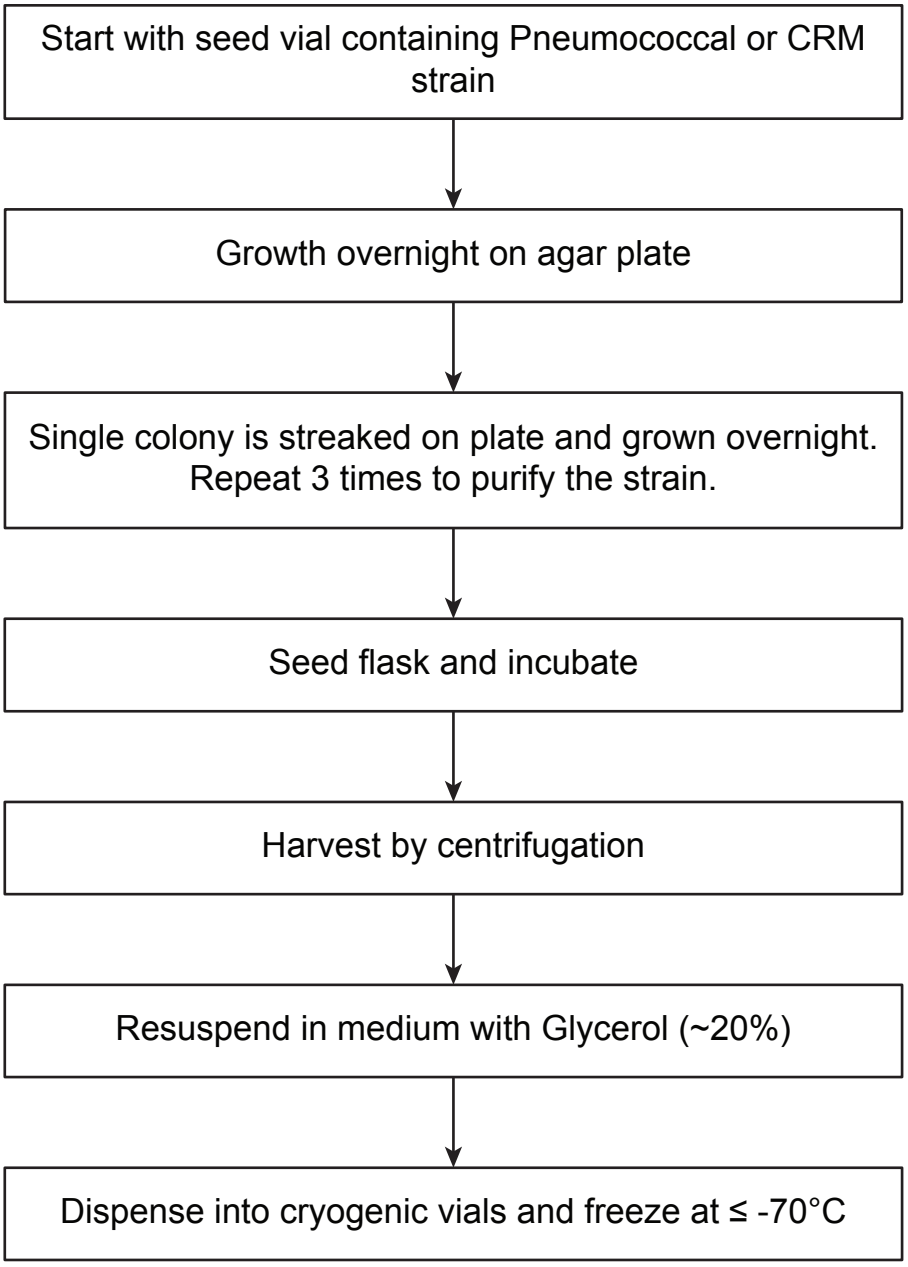

Start with seed vial containing Pneumococcal or CRM strain
Growth overnight on agar plate
Single colony is streaked on plate and grown overnight. Repeat 3 times to purify the strain.
Seed flask and incubate
Harvest by centrifugation
Resuspend in medium with Glycerol (~20%)
Dispense into cryogenic vials and freeze at ≤ -70°C

FIG. 2

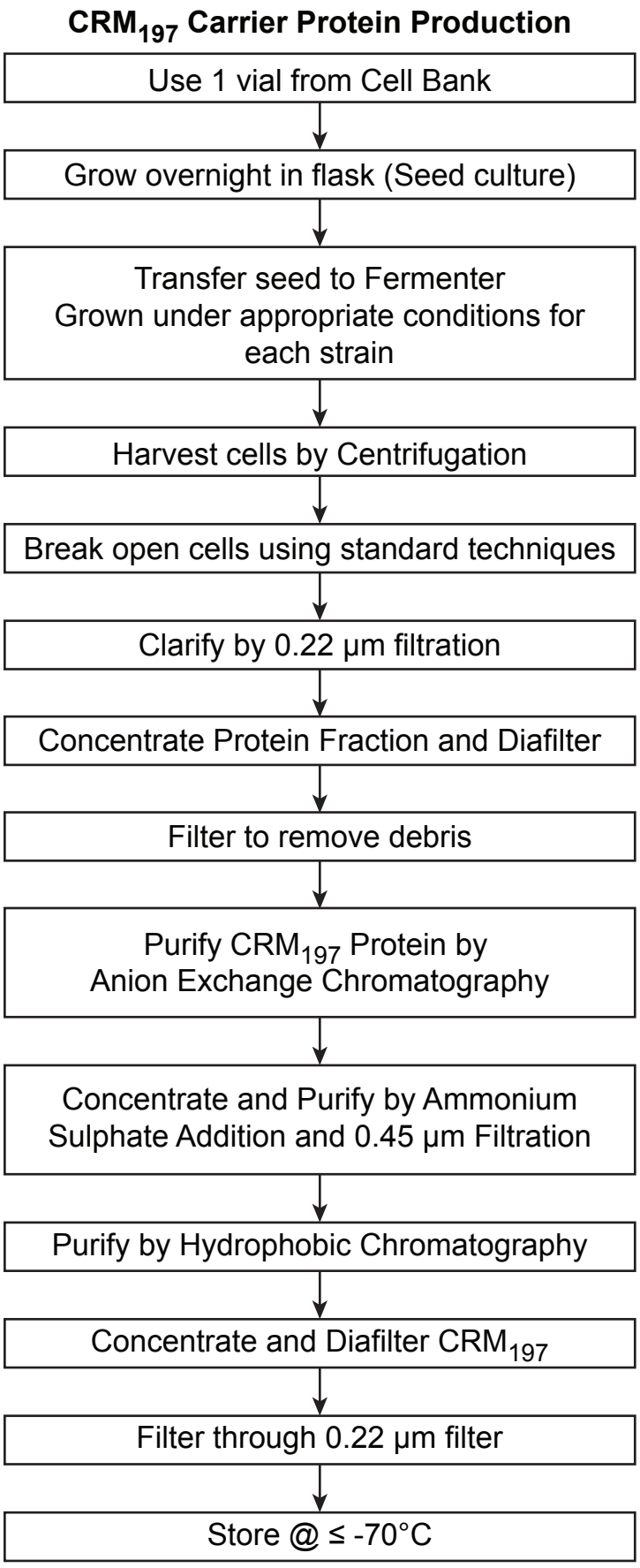

CRM197 Carrier Protein Production
Use 1 vial from Cell Bank
Grow overnight in flask (Seed culture)
Transfer seed to Fermenter
Grown under appropriate conditions for each strain
Harvest cells by Centrifugation
Break open cells using standard techniques
Clarify by 0.22 µm filtration
Concentrate Protein Fraction and Diafilter
Filter to remove debris
Purify CRM197 Protein by Anion Exchange Chromatography
Concentrate and Purify by Ammonium Sulphate Addition and 0.45 µm Filtration
Purify by Hydrophobic Chromatography
Concentrate and Diafilter CRM197
Filter through 0.22 µm filter
Store @ ≤ -70°C

FIG. 3

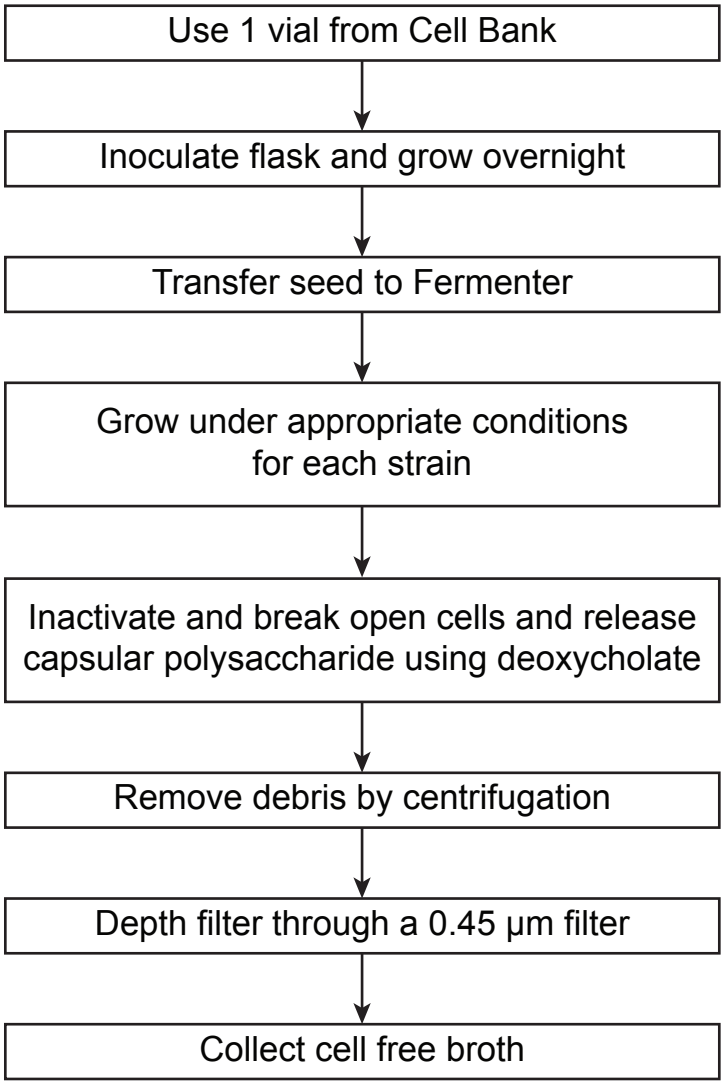

Use 1 vial from Cell Bank
Inoculate flask and grow overnight
Transfer seed to Fermenter
Grow under appropriate conditions for each strain
Inactivate and break open cells and release capsular polysaccharide using deoxycholate
Remove debris by centrifugation
Depth filter through a 0.45 μm filter
Collect cell free broth

FIG. 4

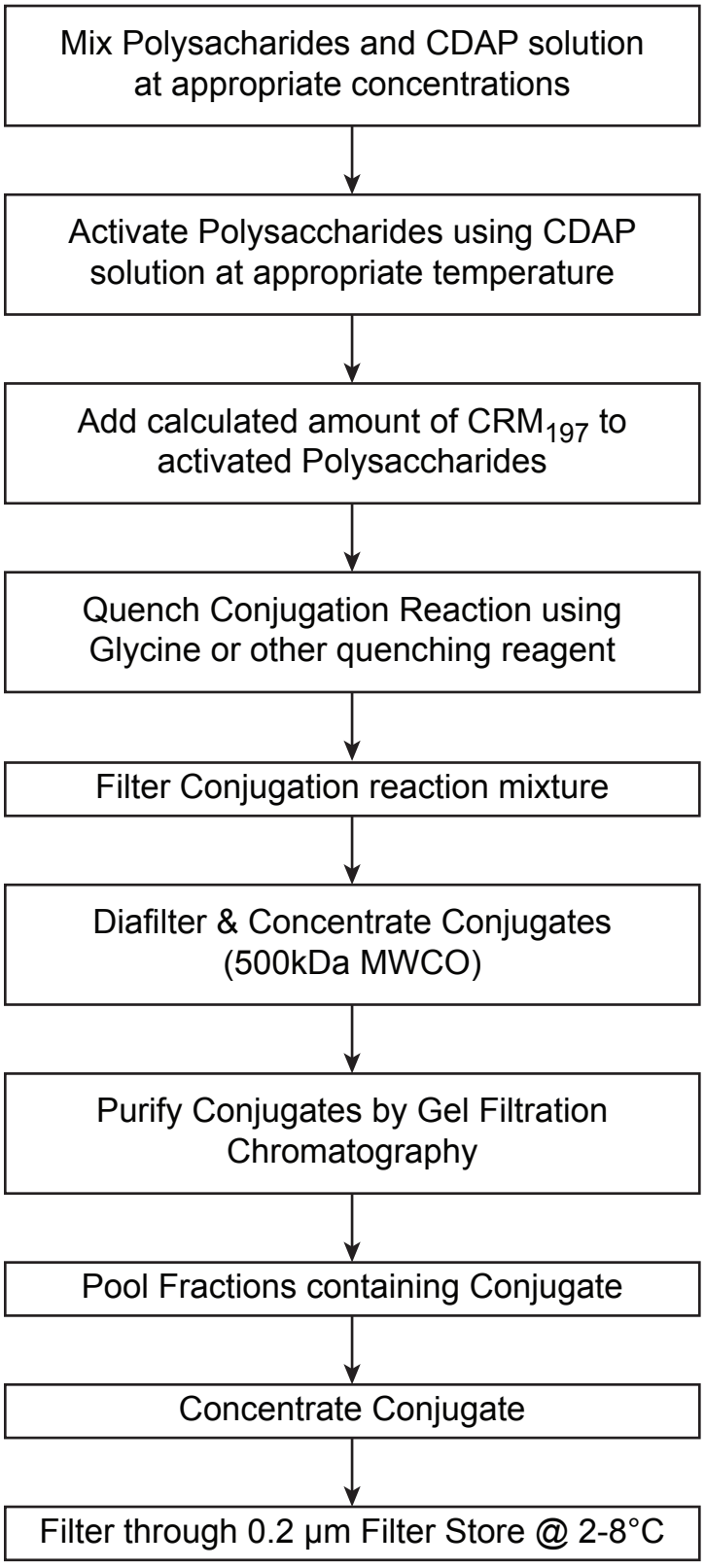

Mix Polysacharides and CDAP solution at appropriate concentrations
Activate Polysaccharides using CDAP solution at appropriate temperature
Add calculated amount of CRM_{197} to activated Polysaccharides
Quench Conjugation Reaction using Glycine or other quenching reagent
Filter Conjugation reaction mixture
Diafilter & Concentrate Conjugates (500kDa MWCO)
Purify Conjugates by Gel Filtration Chromatography
Pool Fractions containing Conjugate
Concentrate Conjugate
Filter through 0.2 µm Filter Store @ 2-8°C

FIG. 5

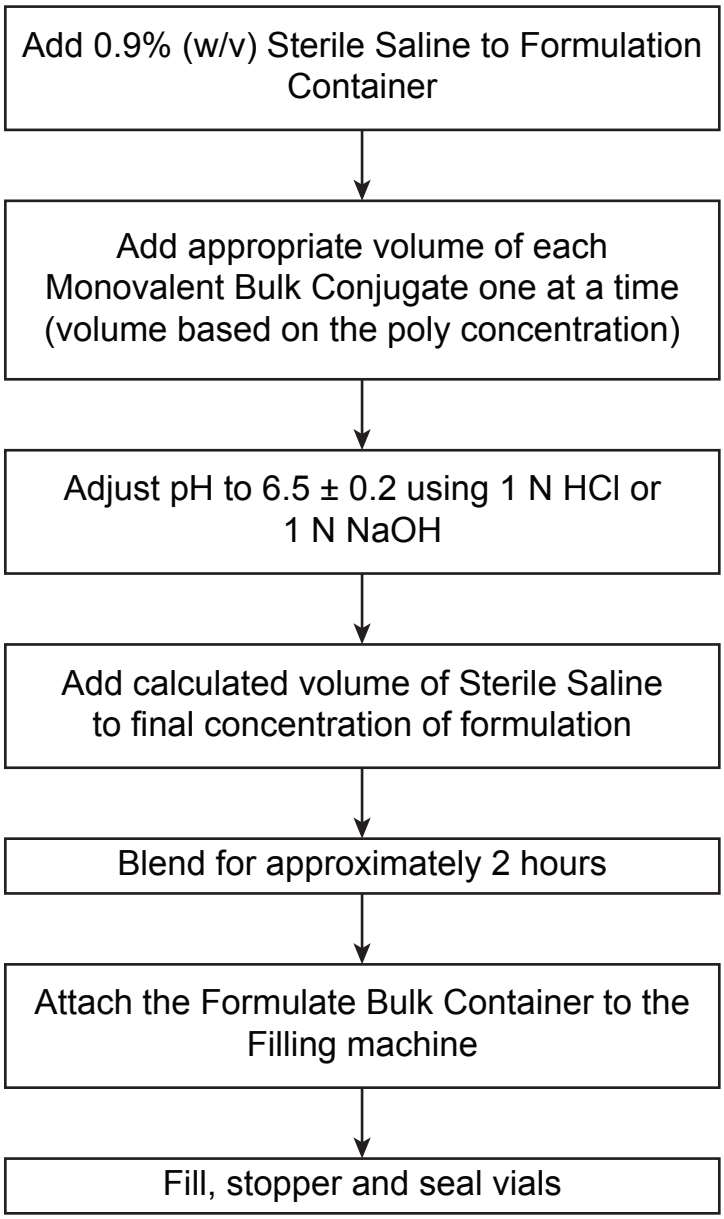

Add 0.9% (w/v) Sterile Saline to Formulation Container
Add appropriate volume of each Monovalent Bulk Conjugate one at a time (volume based on the poly concentration)
Adjust pH to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH
Add calculated volume of Sterile Saline to final concentration of formulation
Blend for approximately 2 hours
Attach the Formulate Bulk Container to the Filling machine
Fill, stopper and seal vials

FIG. 6

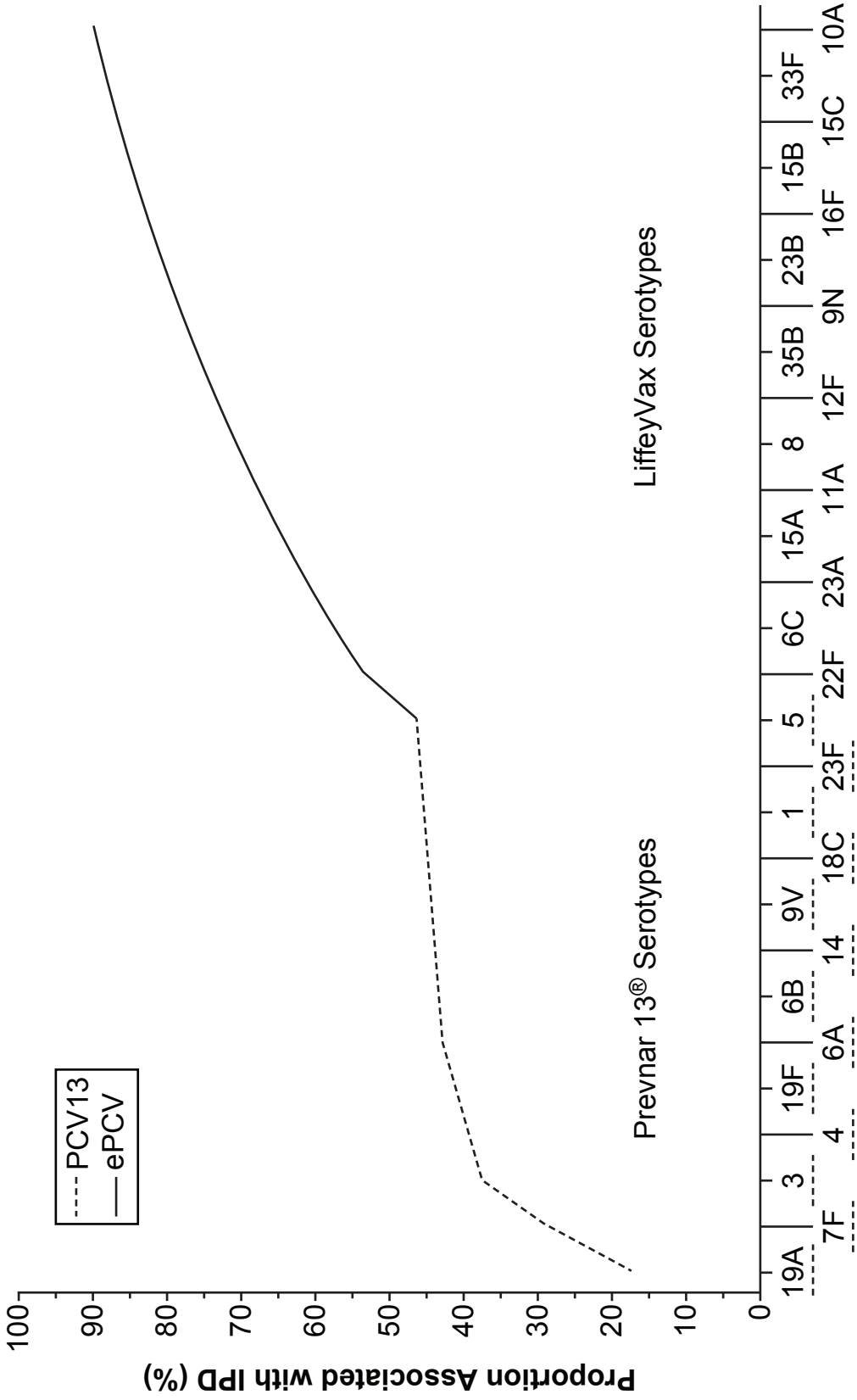

PCV13
ePCV
Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13® Serotypes
LiffeyVax Serotypes
19A
7F
3
4
19F
6A
6B
14
9V
18C
1
23F
5
22F
6C
23A
15A
11A
8
12F
35B
9N
23B
16F
15B
15C
33F
10A
S. pneumoniae Serotypes

FIG. 7

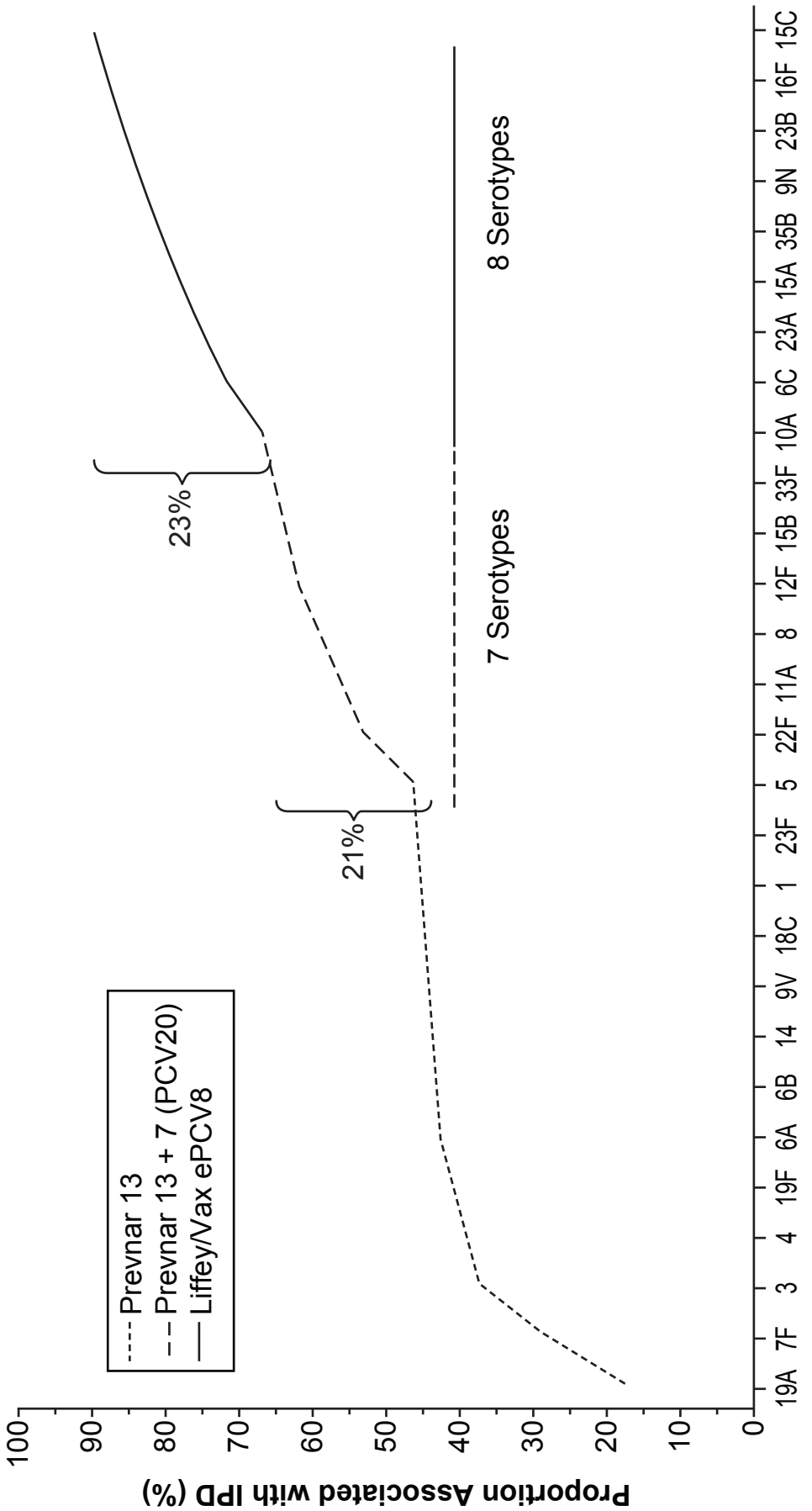

Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13
Prevnar 13 + 7 (PCV20)
Liffey/Vax ePCV8
23%
21%
7 Serotypes
8 Serotypes
19A 7F 3 4 19F 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C
S. pneumoniae Serotypes

FIG. 8

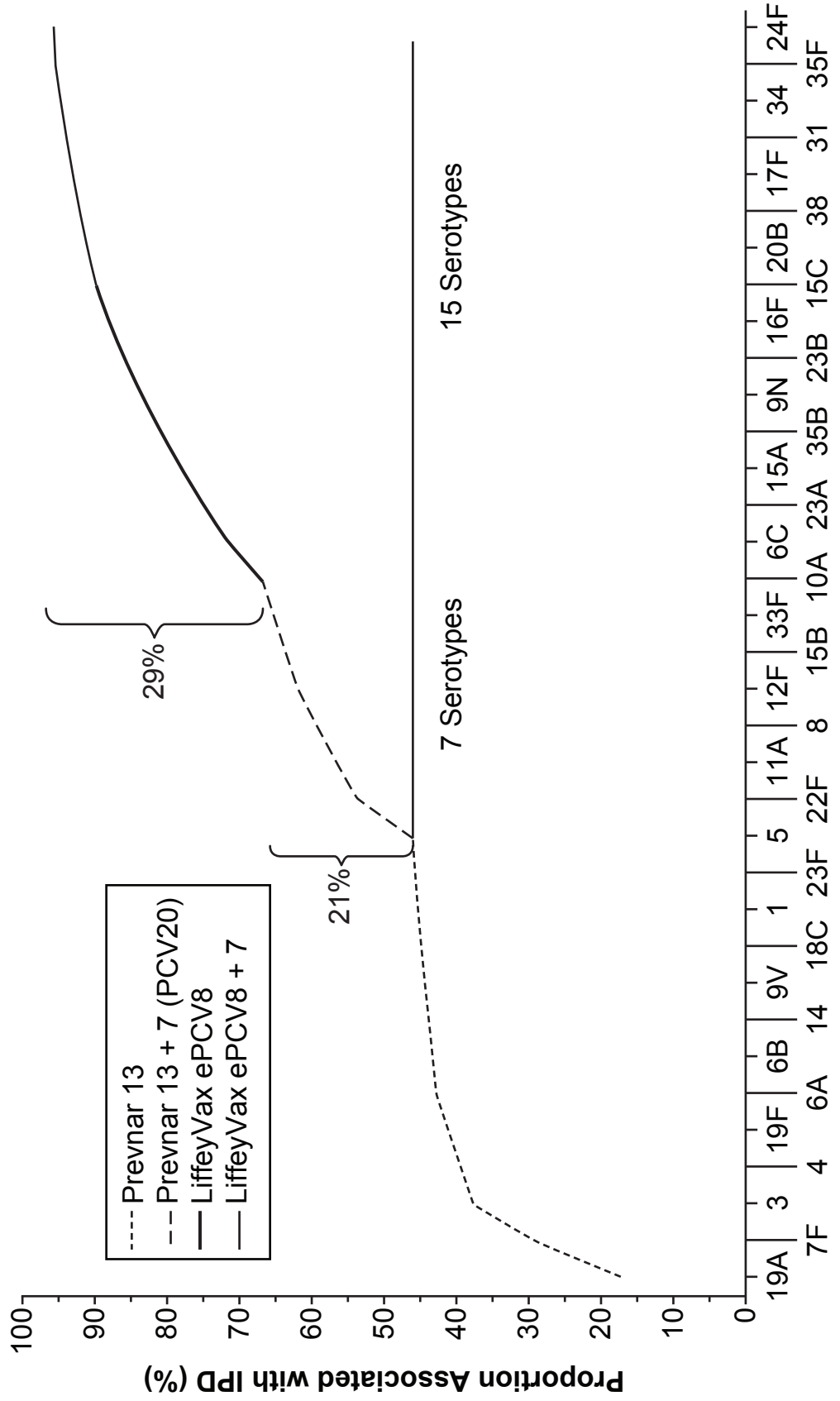

Prevnar 13
Prevnar 13 + 7 (PCV20)
LiffeyVax ePCV8
LiffeyVax ePCV8 + 7
29%
21%
7 Serotypes
15 Serotypes
Proportion Associated with IPD (%)
0
10
20
30
40
50
60
70
80
90
100
19A 7F 3 4 19F 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C 20B 38 17F 31 34 35F 24F
S. pneumoniae Serotypes

FIG. 9

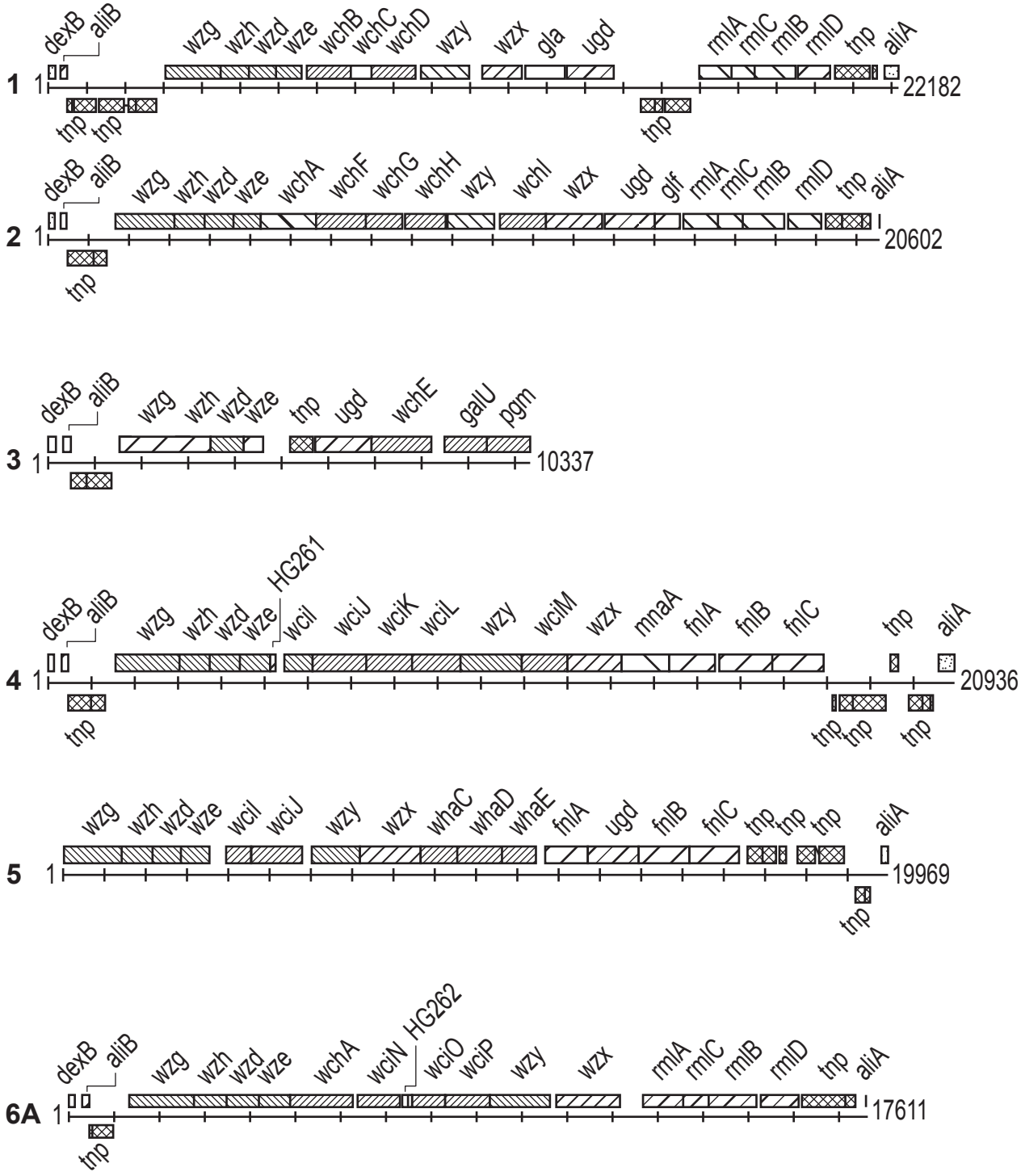

1
dexB
aliB
wzg
wzh
wzd
wze
wchB
wchC
wchD
wzy
wzx
gla
ugd
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
22182
tnp
tnp
tnp
2
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wchG
wchH
wzy
wchI
wzx
ugd
glf
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
20602
tnp
3
dexB
aliB
wzg
wzh
wzd
wze
tnp
ugd
wchE
galU
pgm
1
10337
4
HG261
dexB
aliB
wzg
wzh
wzd
wze
wcil
wciJ
wciK
wciL
wzy
wciM
wzx
mnaA
fnlA
fnlB
fnlC
tnp
aliA
1
20936
tnp
tnp
tnp
tnp
5
wzg
wzh
wzd
wze
wcil
wciJ
wzy
wzx
whaC
whaD
whaE
fnlA
ugd
fnlB
fnlC
tnp
tnp
tnp
aliA
1
19969
tnp
6A
HG262
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciN
wciO
wciP
wzy
wzx
rmlA
rmlC
rmlB
rmlD
tnp
aliA
1
17611
tnp

FIG. 10A

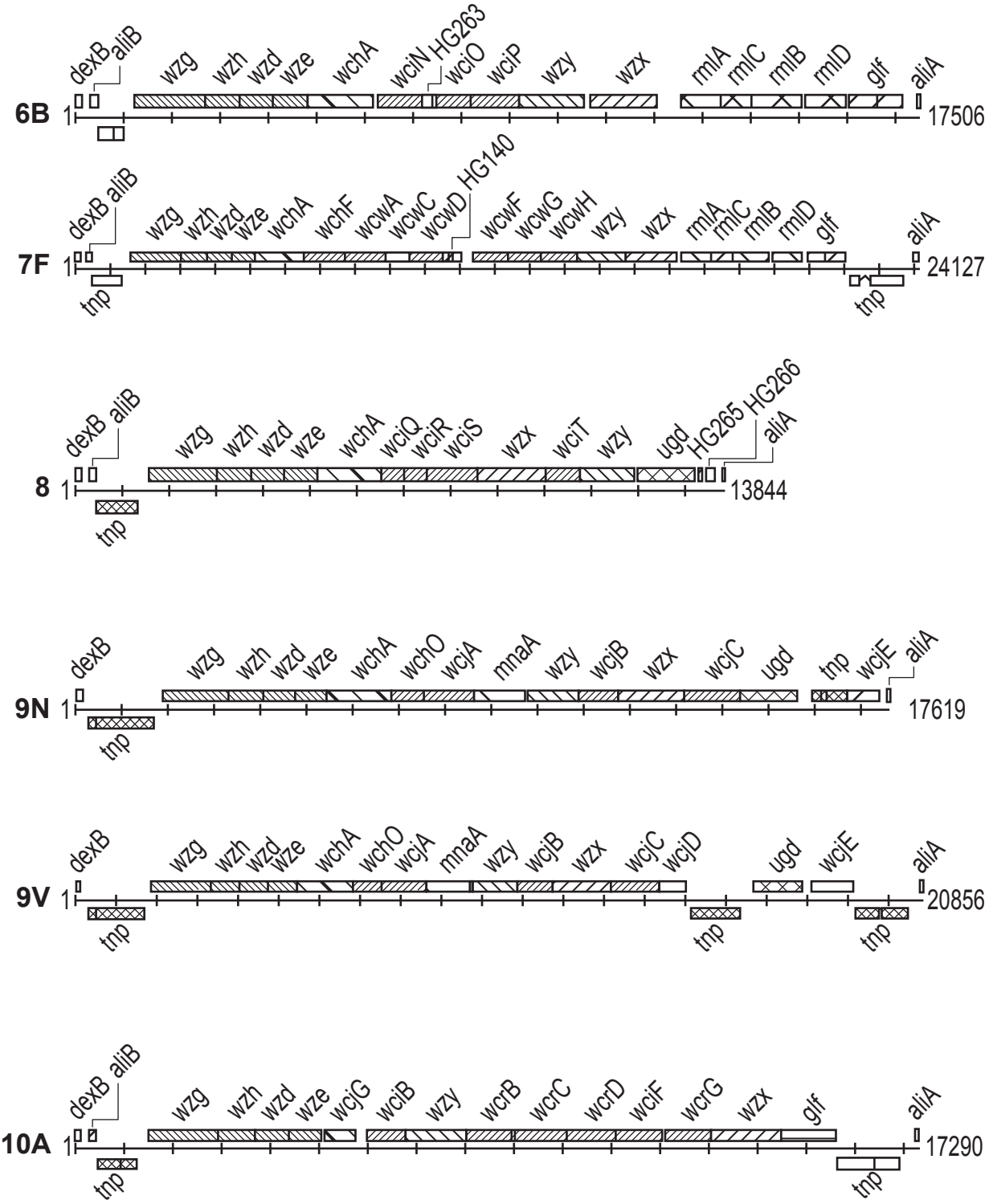

6B
dexB aliB wzg wzh wzd wze wchA wciN HG263 wciO wciP wzy wzx rmlA rmlC rmlB rmlD glf aliA
1
17506
7F
dexB aliB wzg wzh wzd wze wchA wchF wcwA wcwC wcwD HG140 wcwF wcwG wcwH wzy wzx rmlA rmlC rmlB rmlD glf aliA
1
24127
tnp
tnp
8
dexB aliB wzg wzh wzd wze wchA wciQ wciR wciS wzx wciT wzy ugd HG265 HG266 aliA
1
13844
tnp
9N
dexB wzg wzh wzd wze wchA wchO wcjA mnaA wzy wcjB wzx wcjC ugd tnp wcjE aliA
1
17619
tnp
9V
dexB wzg wzh wzd wze wchA wchO wcjA mnaA wzy wcjB wzx wcjC wcjD ugd wcjE aliA
1
20856
tnp
tnp
tnp
10A
dexB aliB wzg wzh wzd wze wcjG wciB wzy wcrB wcrC wcrD wciF wcrG wzx glf aliA
1
17290
tnp
tnp

FIG. 10B

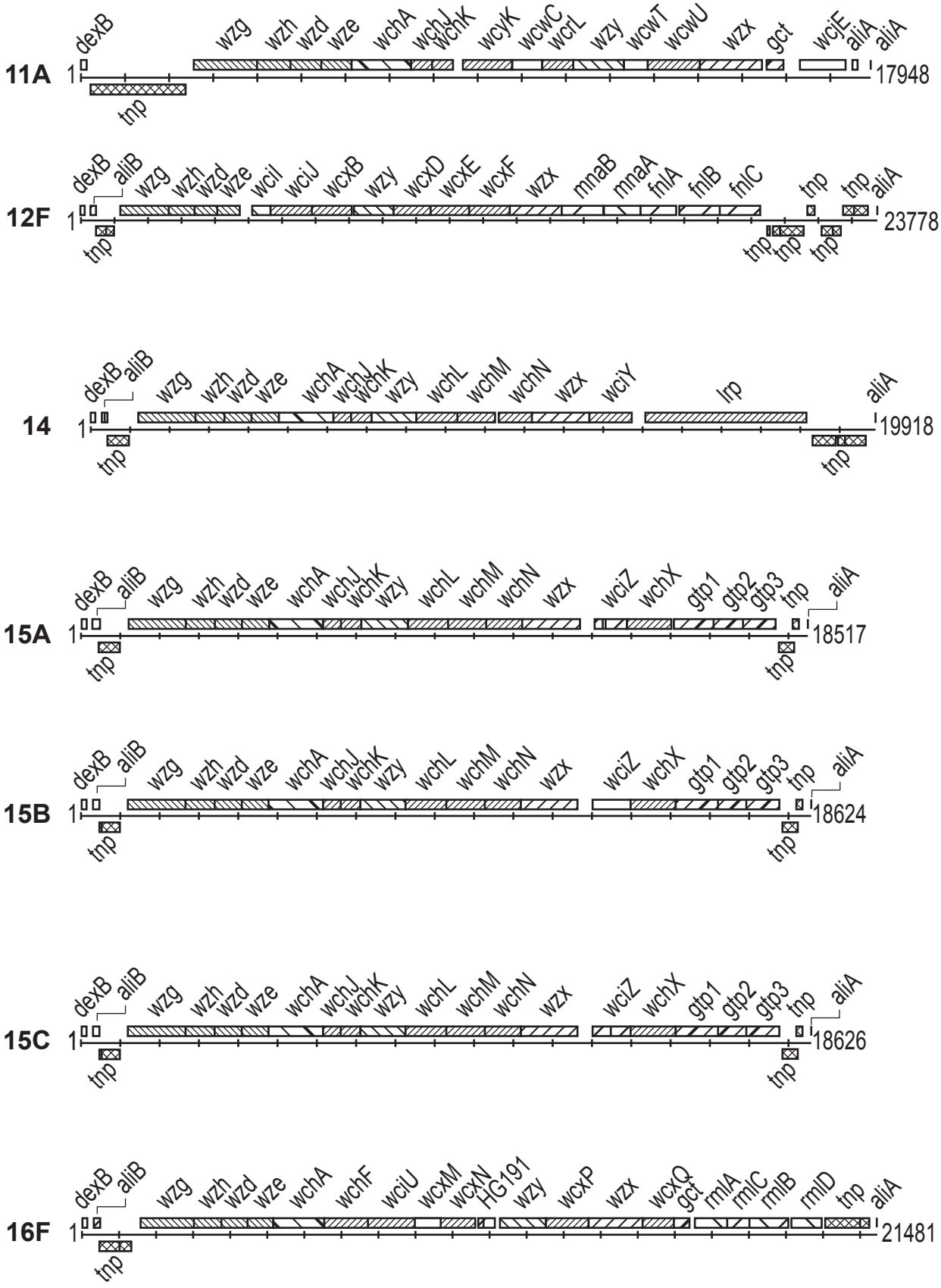

11A
dexB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wcyK
wcwC
wcrL
wzy
wcwT
wcwU
wzx
gct
wciE
aliA
aliA
1
17948
tnp
12F
dexB
aliB
wzg
wzh
wzd
wze
wcil
wciJ
wcxB
wzy
wcxD
wcxE
wcxF
wzx
mnaB
mnaA
fnlA
fnlB
fnlC
tnp
tnp
aliA
1
23778
tnp
tnp
tnp
tnp
14
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciY
lrp
aliA
1
19918
tnp
tnp
15A
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
gtp1
gtp2
gtp3
tnp
aliA
1
18517
tnp
tnp
15B
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
gtp1
gtp2
gtp3
tnp
aliA
1
18624
tnp
tnp
15C
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchJ
wchK
wzy
wchL
wchM
wchN
wzx
wciZ
wchX
gtp1
gtp2
gtp3
tnp
aliA
1
18626
tnp
tnp
16F
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wciU
wcxM
wcxN
HG191
wzy
wcxP
wzx
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gct
rmlA
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rmlD
tnp
aliA
1
21481
tnp

FIG. 10C

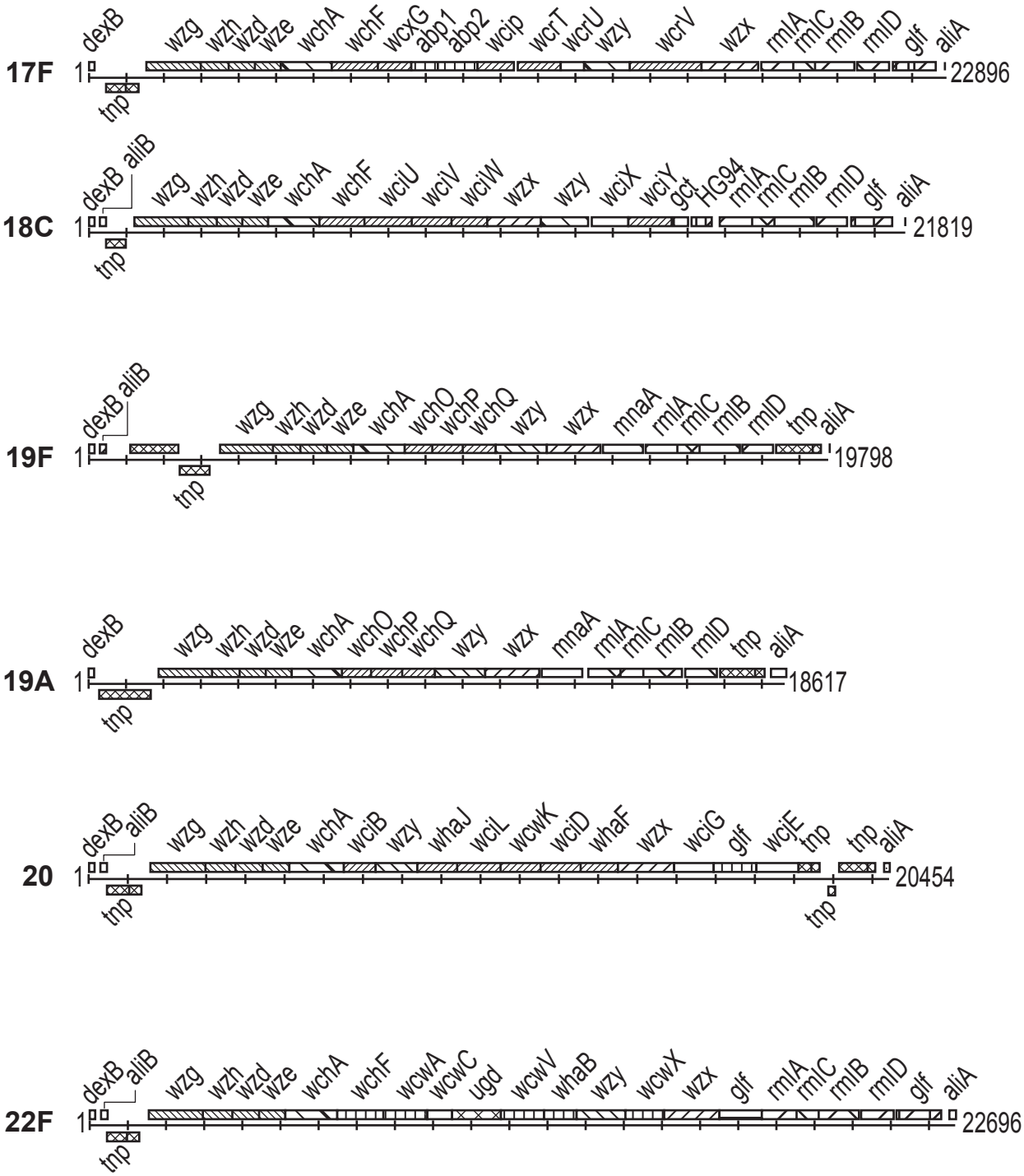

17F
dexB wzg wzh wzd wze wchA wchF wcxG abp1 abp2 wcip wcrT wcrU wzy wcrV wzx rmlA rmlC rmlB rmlD glf aliA
1
22896
tnp
18C
dexB aliB wzg wzh wzd wze wchA wchF wciU wciV wciW wzx wzy wciX wciY gct HG94 rmlA rmlC rmlB rmlD glf aliA
1
21819
tnp
19F
dexB aliB wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx mnaA rmlA rmlC rmlB rmlD tnp aliA
1
19798
tnp
19A
dexB wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx mnaA rmlA rmlC rmlB rmlD tnp aliA
1
18617
tnp
20
dexB aliB wzg wzh wzd wze wchA wciB wzy whaJ wciL wcwK wciD whaF wzx wciG glf wcjE tnp tnp aliA
1
20454
tnp
tnp
22F
dexB aliB wzg wzh wzd wze wchA wchF wcwA wcwC ugd wcwV whaB wzy wcwX wzx glf rmlA rmlC rmlB rmlD glf aliA
1
22696
tnp

FIG. 10D

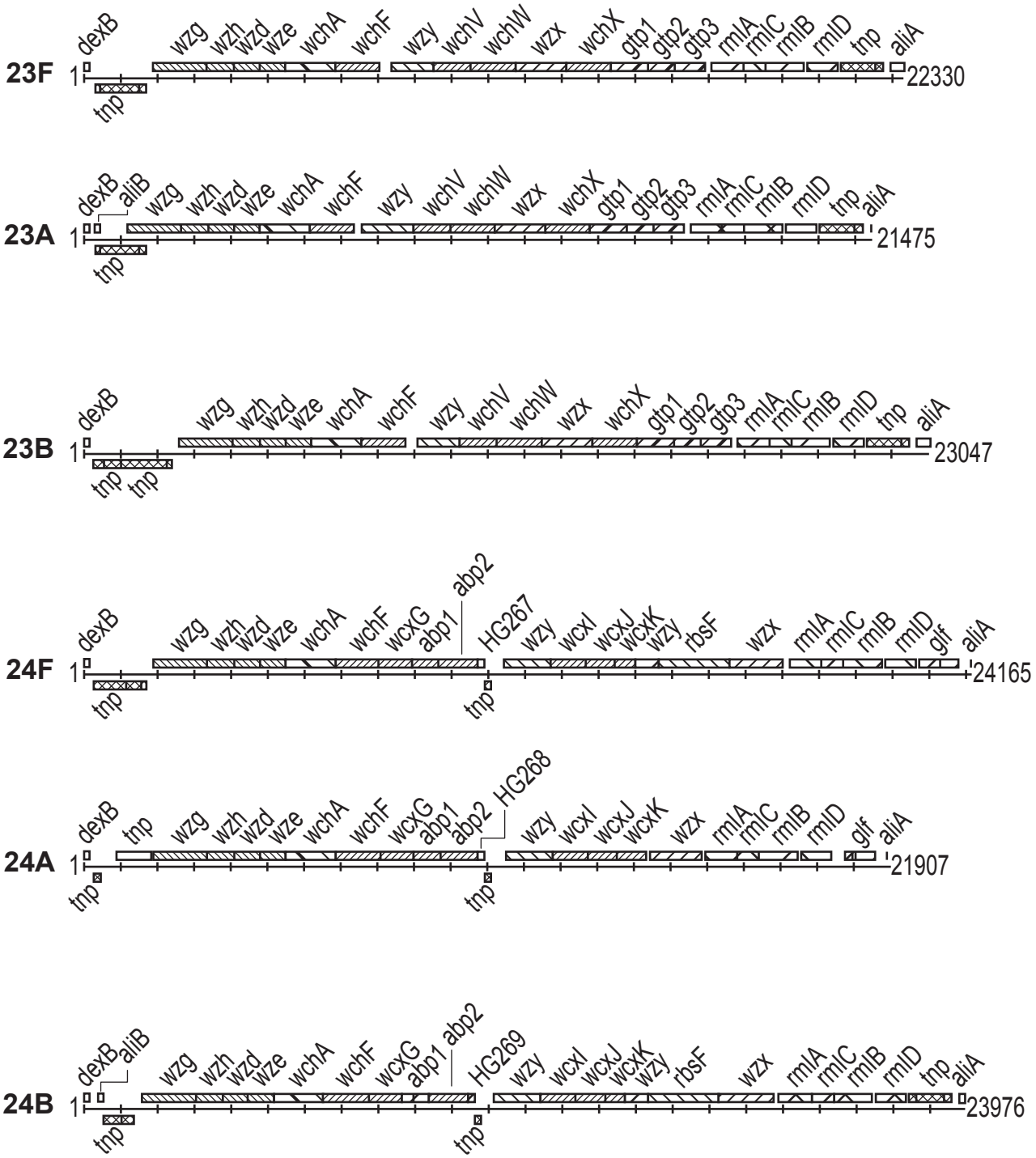

23F
1
dexB
wzg
wzh
wzd
wze
wchA
wchF
wzy
wchV
wchW
wzx
wchX
gtp1
gtp2
gtp3
rmlA
rmlC
rmlB
rmlD
tnp
aliA
22330
tnp
23A
1
dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wzy
wchV
wchW
wzx
wchX
gtp1
gtp2
gtp3
rmlA
rmlC
rmlB
rmlD
tnp
aliA
21475
tnp
23B
1
dexB
wzg
wzh
wzd
wze
wchA
wchF
wzy
wchV
wchW
wzx
wchX
gtp1
gtp2
gtp3
rmlA
rmlC
rmlB
rmlD
tnp
aliA
23047
tnp
tnp
24F
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dexB
wzg
wzh
wzd
wze
wchA
wchF
wcxG
abp1
abp2
HG267
wzy
wcxI
wcxJ
wcxK
wzy
rbsF
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
24165
tnp
tnp
24A
1
dexB
tnp
wzg
wzh
wzd
wze
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wchF
wcxG
abp1
abp2
HG268
wzy
wcxI
wcxJ
wcxK
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
21907
tnp
tnp
24B
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dexB
aliB
wzg
wzh
wzd
wze
wchA
wchF
wcxG
abp1
abp2
HG269
wzy
wcxI
wcxJ
wcxK
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rbsF
wzx
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rmlC
rmlB
rmlD
tnp
aliA
23976
tnp
tnp

FIG. 10E

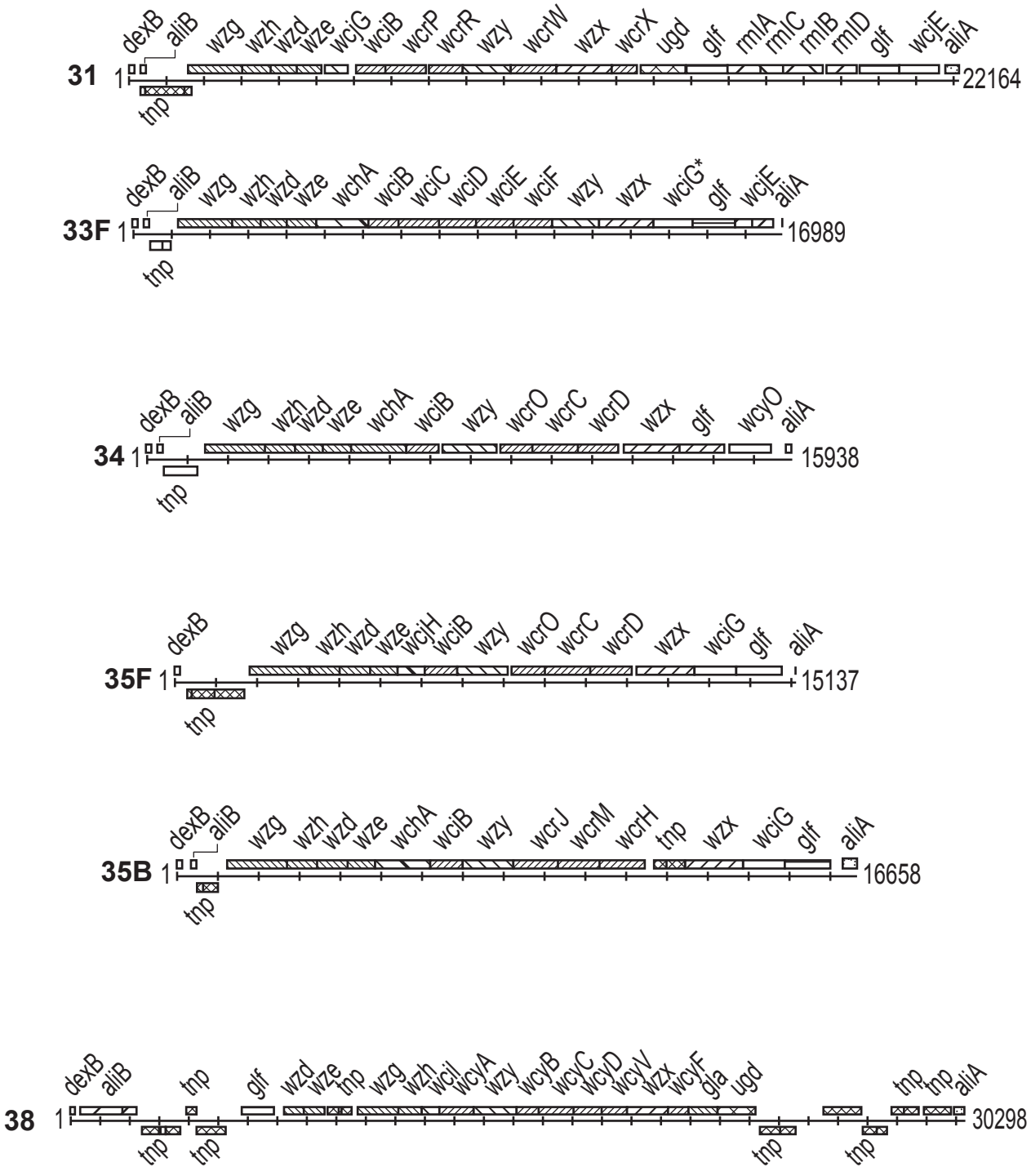

31
1
22164
dexB
aliB
wzg
wzh
wzd
wze
wcjG
wciB
wcrP
wcrR
wzy
wcrW
wzx
wcrX
ugd
glf
rmlA
rmlC
rmlB
rmlD
glf
wcjE
aliA
tnp
33F
1
16989
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wciC
wciD
wciE
wciF
wzy
wzx
wciG*
glf
wcjE
aliA
tnp
34
1
15938
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wzy
wcrO
wcrC
wcrD
wzx
glf
wcyO
aliA
tnp
35F
1
15137
dexB
wzg
wzh
wzd
wze
wciH
wciB
wzy
wcrO
wcrC
wcrD
wzx
wciG
glf
aliA
tnp
35B
1
16658
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wzy
wcrJ
wcrM
wcrH
tnp
wzx
wciG
glf
aliA
tnp
38
1
30298
dexB
aliB
tnp
glf
wzd
wze
tnp
wzg
wzh
wciI
wcyA
wzy
wcyB
wcyC
wcyD
wcyV
wzx
wcyF
gla
ugd
tnp
tnp
aliA
tnp
tnp
tnp
tnp

FIG. 10F

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Application Number: 17347435 Document Date: 06/14/2021

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MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
17347435	

Applicant(s) Bruce Forrest

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
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100		(1)				

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
17347435	

Applicant(s) Bruce Forrest

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
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149		(1)				
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	*		*		*	
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152		(1)				
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MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
17347435	

Applicant(s) Bruce Forrest

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
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204		(1)				
205		(1)				
206		(1)				
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248		(1)				
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250		(1)				
Total Indep	6		0		0	
Total Depend	313	↵	0	↵	0	↵
Total Claims	319		0		0	

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
251		(1)				
252		(1)				
253		(1)				
254		(1)				
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299						
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 17/347,435

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	80		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	350		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	400		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	319 minus 20 =	* 299	x 50 =	14950	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3	x 240 =	720			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				430			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	16930		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/347,435	06/14/2021	Bruce D. Forrest	P1143.70000US05

CONFIRMATION NO. 4020

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

FORMALITIES LETTER



Date Mailed: 06/30/2021

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Items 1) and 2) provide general guidance related to requirements for sequence disclosures.

1) 37 CFR 1.821(c) requires that patent applications which contain disclosures of nucleotide and/or amino acid sequences that fall within the definitions of 37 CFR 1.821(a) must contain a "Sequence Listing", *as a separate part of the disclosure*, which presents the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of 37 CFR 1.821-1.825. This "Sequence Listing" part of the disclosure may be submitted:
   a) via EFS-Web (see Section I.1 of the Legal Framework for EFS-Web (https://www.uspto.gov/patents-application-process/filing-online/legal-framework-efs-web), hereinafter "Legal Framework") as an ASCII text file, together with an incorporation-by-reference of the material in the ASCII text file in a separate paragraph of the specification identifying:
      i) the name of the ASCII text file;
      ii) the date of creation; and
      iii) the size of the ASCII text file in bytes;
   b) on compact disc(s) in duplicate according to 37 CFR 1.52(e)(1)(ii) and (4), labeled according to 37 CFR 1.52(e)(6), together with a statement that the duplicate compact discs are identical and an incorporation-by-reference of the material in the ASCII text file according to 37 CFR 1.52(e)(5) in a separate paragraph of the specification identifying:
      i) the name of the ASCII text file;
      ii) the date of creation; and
      iii) the size of the ASCII text file in bytes;
   c) via EFS-Web as a PDF (not recommended); or
   d) on paper.
2) 37 CFR 1.821(e) requires that a *copy* of the "Sequence Listing" must also be submitted in computer readable form (CRF) in accordance with the requirements of 37 CFR 1.824.

Petitioner Merck Ex-1015, 0167

a) If a "Sequence Listing" ASCII text file submitted via EFS-Web on the application filing date complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b), and applicant has not filed a "Sequence Listing" as a PDF, the text file will serve as both the "Sequence Listing" required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e), and the statement of identity under the "Legal Framework" is not required.
b) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed via EFS-Web as a PDF, then the "Legal Framework" requires submission of a statement that the "Sequence Listing" content of the PDF copy and the ASCII text file copy submitted via EFS-Web are identical.
c) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed on paper or compact disc, then 37 CFR 1.821(f) requires submission of a statement that the "Sequence Listing" content of the paper or compact disc copy and the CRF are identical.

Specific deficiencies and the required response to this notice are as follows:

- This application fails to comply with the requirements of 37 CFR 1.821-1.825 because it does not contain a "Sequence Listing" as a separate part of the disclosure or a CRF. Applicant must provide:
  - A "Sequence Listing" part of the disclosure, as described above in item 1); **together with**
  - **An amendment specifically directing its entry into the application;**
  - A statement that the "Sequence Listing" includes no new matter; and
  - A statement that indicates support for the amendment in the application, as filed, as required by 37 CFR 1.825 and the "Legal Framework".
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) a) or b) above, Applicant must also provide:
  - A substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125 inserting the required incorporation-by-reference paragraph, consisting of:
    - A copy of the previously-submitted specification, with deletions shown with strikethrough or brackets and insertions shown with underlining (marked-up version);
    - A copy of the amended specification without markings (clean version); and
    - A statement that the substitute specification contains no new matter.
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) b), c), or d) above, Applicant must also provide:
  - A CRF; and
  - A statement according to item 2) b) or c) above.
- If applicant desires the CRF in this application to be identical with the CRF of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF (Form PTO/SB/93 may be used for this purpose).

For questions regarding compliance to these requirements, please contact:

- **For Rules interpretation, call (571) 272-2510**
- **For CRF submission help, call (571) 272-2510**
- **For PatentIn software program support technical assistance, call (866) 217-9197.**
- **Send e-mail correspondence for PatentIn software program help to ebc@uspto.gov**
- **PatentIn software is available at http://www.uspto.gov/patents/resources/tools/checker/patentinrel.jsp .**

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

- The statutory basic filing fee is missing.

Petitioner Merck Ex-1015, 0168

- The application search fee must be submitted.
- The application examination fee must be submitted.
- Additional claim fees of $ **16100** as a small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. Small entity discount is in effect. If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **80** basic filing fee.
- $ **80** surcharge.
- $ **350** search fee.
- $ **400** examination fee.
- $ **720** for **3** independent claims over 3.
- $ **14950** for **299** total claims over 20.
- $ **430** for multiple dependent claim surcharge.
- $(**0**) previous unapplied payment amount.
- $ **17010** TOTAL FEE BALANCE DUE.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

Petitioner Merck Ex-1015, 0169

/ygizaw/

Petitioner Merck Ex-1015, 0170

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
17/347,435	06/14/2021		0.00	P1143.70000US05	295	6

CONFIRMATION NO. 4020

FILING RECEIPT

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206



Date Mailed: 06/30/2021

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Bruce D. Forrest, South Nyack, NY;
Jack D. Love, Tampa, FL;

Applicant(s)

Pogona, LLC, South Nyack, NY;

Assignment For Published Patent Application

Pogona, LLC, South Nyack, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/089,040 09/27/2018 PAT 11058757
which is a 371 of PCT/US2017/025621 03/31/2017
which claims benefit of 62/373,807 08/11/2016
and claims benefit of 62/372,263 08/08/2016
and claims benefit of 62/330,245 05/02/2016
and claims benefit of 62/316,555 03/31/2016

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Petitioner Merck Ex-1015, 0171

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 06/24/2021

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/347,435**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Petitioner Merck Ex-1015, 0174

Docket No.: P1143.70000US05
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	17/347,435
Confirmation No.:	4020
Filed:	June 14, 2021
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: January 28, 2022 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

In response to the Notice to File Missing Parts of Nonprovisional Application, mailed June 30, 2021, Applicant submits herewith a Preliminary Amendment. Applicant requests that the excess claim fees, if applicable, be calculated after entrance of the Preliminary Amendment.

The deadline for responding to the Notice was August 30, 2021. A five-month extension of time from August 30, 2021, up to and including January 30, 2022, is hereby respectfully requested, and the extension fee is being paid using the credit card payment option of the USPTO's Electronic Filing System. Applicant thus submits that the present Response is timely submitted on January 28, 2022.

Petitioner Merck Ex-1015, 0175

Please charge any deficiency in the fees due, or credit any overpayment, to our Deposit Account No. 23/2825, under Docket No. P1143.70000US05, from which the undersigned is authorized to draw.

Dated: January 28, 2022

Respectfully submitted,

By: /Andrew W. Williams/
Andrew W. Williams, PhD
Registration No.: 48,644
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US05
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	17/347,435
Confirmation No.:	4020
Filed:	June 14, 2021
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: January 28, 2022 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

SECOND PRELIMINARY AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Prior to examination on the merits, please amend the above-referenced U.S. Patent Application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 3 of this paper.

Remarks begin on page 6 of this paper.

Petitioner Merck Ex-1015, 0177

AMENDMENTS TO THE SPECIFICATION

Sequence Listing

Please insert the Sequence Listing submitted herewith in computer readable form (.txt file) into the above-referenced Application immediately before the claims and renumber the subsequent pages accordingly. No new matter has been added.

Substitute Specification

Please replace the specification on record with the Substitute Specification filed with this Response in accordance with 37 CFR § 1.125. The specification has been amended to include a paragraph to reference the submission of sequences listing via EFS-web. No new matter has been added. Two versions of the Substitute Specification are submitted: (1) a marked-up version showing additions by underlining and deletions by strike-through or double brackets; and (2) a clean version without markings. Applicant submits that no new matter is added to the Application by this Substitute Specification.

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, and listings, of claims in the Application:

Listing of Claims

1-295. (Cancelled)

296. (New) 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 a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 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, wherein the at least one additional serotype is not the first serotype.

297. (New) The pharmaceutical composition of claim 296, wherein the first serotype is 20B.

298. (New) The pharmaceutical composition of claim 296, wherein the first serotype is 6C.

299. (New) The pharmaceutical composition of claim 296, wherein the first serotype is 15A.

300. (New) The pharmaceutical composition of claim 296, wherein the first serotype is 16F.

301. (New) The pharmaceutical composition of claim 296, wherein at least one polypeptide comprises a mixture of polypeptides.

302. (New) The pharmaceutical composition of claim 296, wherein at least one 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, PspA, any fragment thereof, or any combination thereof.

303. (New) The pharmaceutical composition of claim 296, further comprising an adjuvant; a chelating agent; a surfactant; an emulsifier; a buffering agent; a preservative; a salt; an anti-fungal compound; or a combination thereof.

304. (New) The pharmaceutical composition of claim 296, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

305. (New) The pharmaceutical composition of claim 296, wherein at least one of the immunogenic saccharide-polypeptide conjugates elicits an opsonophagocytic response.

306. (New) The pharmaceutical composition of claim 296, wherein the immunogenic saccharide-polypeptide conjugates are collectively present in an amount of at least 0.001%, by weight, based on the weight of the pharmaceutical composition.

307. (New) The pharmaceutical composition of claim 296, wherein the immunogenic saccharide-polypeptide conjugates comprise: (i) the capsular polysaccharide at least partially embedded in the polypeptide, (ii) the capsular polysaccharide chemically cross-linked to the polypeptide, and/or (iii) the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

308. (New) The pharmaceutical composition of claim 296, wherein a toxin activity of at least one of the polypeptides is at least partly mitigated.

309. (New) The pharmaceutical composition of claim 296, wherein each of the immunogenic saccharide-polypeptide conjugates is present in an amount of at least 0.001%, by weight, based on the weight of the pharmaceutical composition.

310. (New) A method comprising administering to a subject a first composition, wherein the first composition comprises the pharmaceutical composition of claim 296.

311. (New) The method of claim 310, wherein the first composition is administered intramuscularly.

312. (New) The method of claim 310, 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.

313. (New) The method of claim 312, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide from a serotype of Streptococcus pneumoniae conjugated to a second polypeptide.

314. (New) The method of claim 310, wherein the subject is a human.

315. (New) A method of making a composition comprising: contacting the plurality of immunogenic saccharide-polypeptide conjugates of claim 296 with an excipient, an adjuvant, or any combination thereof.

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of and claims under 35 U.S.C. § 120 to U.S. Patent Application U.S.S.N. 16/089,040, filed September 27, 2018, which is a national stage filing under 35 U.S.C. § 371 of International PCT Application PCT/US2017/025621, filed March 31, 2017, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application, U.S.S.N. 62/316,555, filed on March 31, 2016; U.S. Provisional Application, U.S.S.N. 62/330,245, filed on May 2, 2016; U.S. Provisional Application, U.S.S.N. 62/372,263, filed on August 8, 2016; and U.S. Provisional Application, U.S.S.N. 62/373,807, filed on August 11, 2016, each of which is herein incorporated by reference.

SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 27, 2022, is named P114370000US05-SEQ-AZW and is 5,194 bytes in size.

SUMMARY

[0003] 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.

[0004] 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,

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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 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.

[0005] 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,

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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).

[0006] 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 has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

[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 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] 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.

[0009] Provided herein is a kit comprising of any pharmaceutical composition described herein or any pharmaceutical composition contained in a container described herein.

[0010] Provided herein are methods of making compositions, methods of immunizing with compositions, and methods of vaccinating with compositions.

INCORPORATION BY REFERENCE

[0011] 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.

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BRIEF DESCRIPTION OF THE FIGURES

[0012] 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:

[0013] FIGURE 1 illustrates a flow diagram of the general process for manufacture of a saccharide peptide conjugate vaccine.

[0014] FIGURE 2 illustrates a flow diagram of a process for cell banking of a bacterial strain.

[0015] FIGURE 3 illustrates a flow diagram of a process for producing, isolating, and purifying CRM_{197}.

[0016] FIGURE 4 illustrates a flow diagram of a fermentation process for isolating a polysaccharide from a strain of *Streptococcus pneumoniae*.

[0017] FIGURE 5 illustrates a flow diagram of a conjugation process for conjugating a polysaccharide and CRM_{197}.

[0018] FIGURE 6 illustrates a flow diagram for a process for formulating a polysaccharide polypeptide conjugate vaccine.

[0019] 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.

[0020] 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

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serotypes in the Liffey/Vax ePCV8 is predicted to cover about 23% more of IPD when administered in a vaccination regime with PCV20.

[0021] 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 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.

[0022] 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

[0023] 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.

[0024] 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".

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[0025] 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.

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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

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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%.

[0031] 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.

[0032] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0033] 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}.

[0034] 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.

[0035] 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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[0036] 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.

[0037] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0038] The term "plurality" can mean two or more.

[0039] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

[0040] 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)*.

[0041] 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).

[0042] 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

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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

[0043] 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. 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.

[0044] 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-Pnc*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.

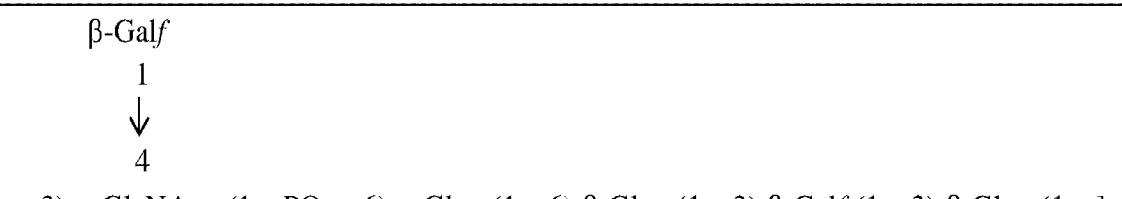
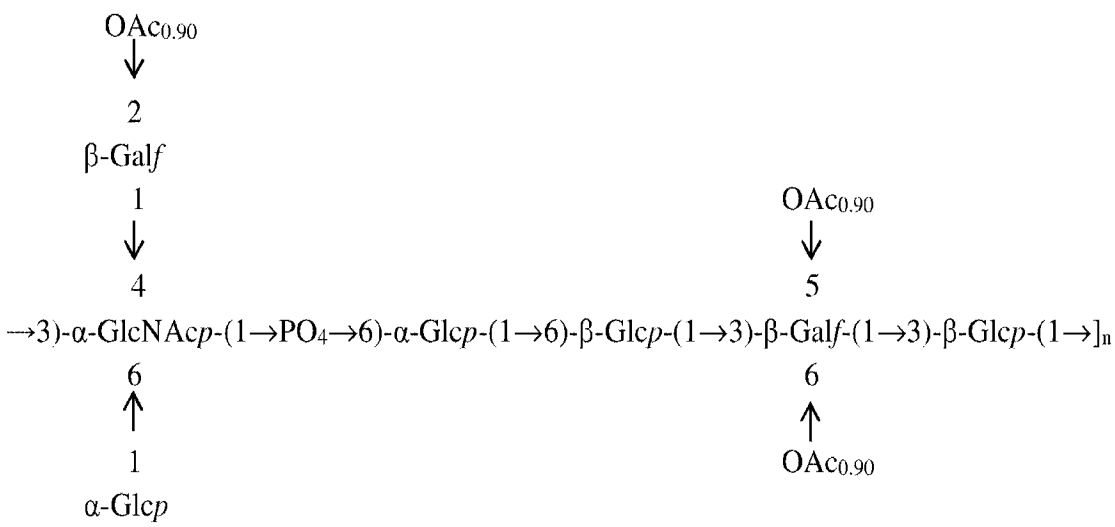
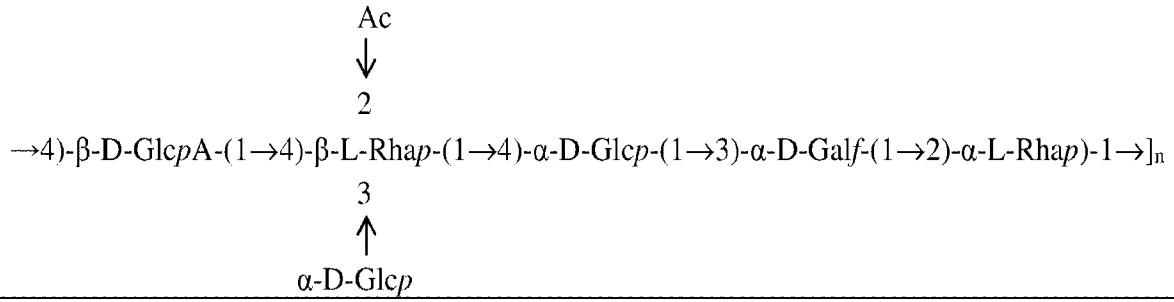
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$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

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Serotype	Polysaccharide Structure (Repeat Unit)
	β-D-Gal*p*
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-PO4$^-$-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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[0045] 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.

[0046] 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.

[0047] 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.

[0048] The saccharides or polysaccharides of the invention are depolymerized (sized) to a final range of 100-500 kDa.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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}.

[0053] 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*.

[0054] 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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[0055] 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

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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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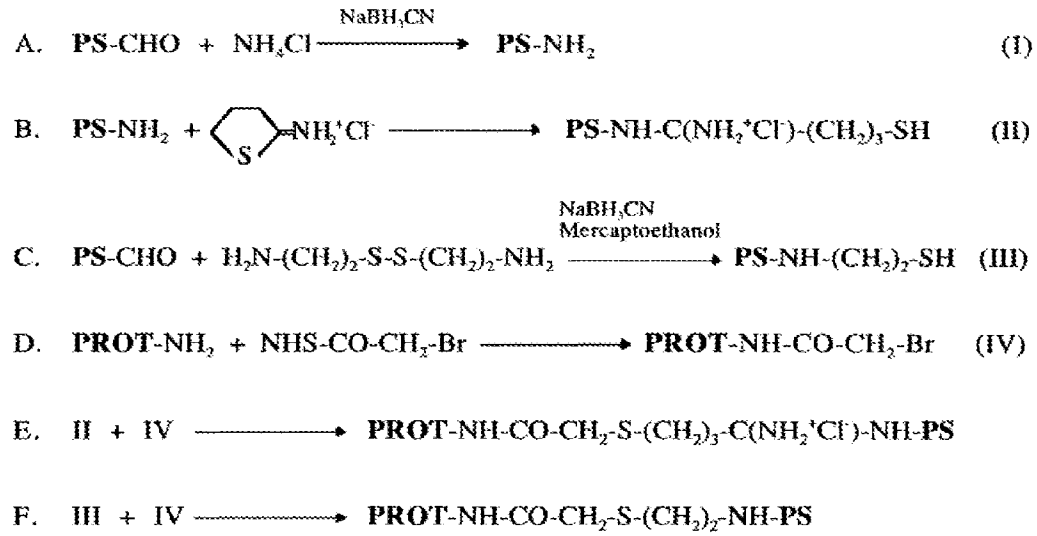
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[0063] 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.

[0064] 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.

[0065] 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

[0066] 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).

[0067] 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.

[0068] 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.

[0069] 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

[0070] 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).

[0071] 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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[0072] 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*.

[0073] 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.

[0074] 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.*, Oxydcoxynucleotides 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.

[0075] 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 Palmitate, 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.

[0076] 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.

[0077] 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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[0078] 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.

[0079] 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

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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*.

[0090] 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*.

[0091] 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.

[0092] 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.

[0093] 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

[0094] 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

[0095] 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

[0096] 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.

[0097] *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.

[0098] *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

[0099] 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).

[0100] 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

[0101] 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

[0102] 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

[0103] 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.

[0104] 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

[0105] 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

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List of Polysaccharide Serotype Combinations
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 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

[0106] 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

[0107] 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

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appropriate volume of each monovalent bulk polysaccharide-CRM_{197} conjugate is added (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

[0108] 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.

[0109] 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

[0110] 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

[0111] 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

[0112] 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

[0113] 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

[0114] 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

[0115] 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

[0116] 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*

[0117] 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.

[0118] 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*

[0119] 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.

[0120] 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*

[0121] 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.

[0122] 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

[0123] 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.

[0124] 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

[0125] 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.

[0126] 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

[0127] 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.

[0128] 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

[0129] 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.

[0130] 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

[0131] 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.

[0132] 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*

[0133] 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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[0134] 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*

[0135] 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.

[0136] 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*

[0137] 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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[0138] 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.

[0139] 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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Electronic Patent Application Fee Transmittal

Application Number:	17347435
Filing Date:	14-Jun-2021
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. Forrest
Filer:	Andrew W. Williams/Chelsea Dowling
Attorney Docket Number:	P1143.70000US05

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	80	80
UTILITY SEARCH FEE	2111	1	350	350
UTILITY EXAMINATION FEE	2311	1	400	400
Pages:				
Claims:				
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	2051	1	80	80
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 5 months with $0 paid	2255	1	1580	1580
Miscellaneous:				
		Total in USD ($)		**2490**

Electronic Acknowledgement Receipt

EFS ID:	44870057
Application Number:	17347435
International Application Number:	
Confirmation Number:	4020
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. Forrest
Customer Number:	23628
Filer:	Andrew W. Williams/Chelsea Dowling
Filer Authorized By:	Andrew W. Williams
Attorney Docket Number:	P1143.70000US05
Receipt Date:	28-JAN-2022
Filing Date:	14-JUN-2021
Time Stamp:	22:07:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$2490
RAM confirmation Number	E20221RM07212351
Deposit Account	232825
Authorized User	Chelsea Dowling

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	P114370000US05-TRN-AZW.pdf	26834 692aa4ab17cdd9c4afa3077eaef9ed260a6670c5	no	1
Warnings:					
Information:					
2	Extension of Time	P114370000US05-EXT-AZW.pdf	25032 a11bc92c0dc21011137d5b957975030ba6d6a784	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	P114370000US05-FEE-AZW.pdf	37452 ecb5389ec94ab7cead22b84c3f0a95e74cd04757	no	1
Warnings:					
Information:					
4	Applicant Response to Pre-Exam Formalities Notice	P114370000US05-NTMP-AZW.pdf	26880 4d95d7c1ca3b986ce8221aa25aa5dede0925f852	no	2
Warnings:					
Information:					
5		P114370000US05-PAMN-AZW.pdf	41699 92db1e0352373bab277371874293a49190f5514e	yes	6

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Specification	2	2
Claims	3	5

	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
6	Specification	P114370000US05-MUSPEC-AZW.pdf	301314 4ec06050f8c492c02dec4b360622abc03e965027	no	87
Warnings:					
Information:					
7	Specification	P114370000US05-SUBSPEC-AZW.pdf	300326 598f599f79cdf562f5770dab1593714a31b884b0	no	87
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	49423 ffc96d95058ec37f1fc5483c282fe053037c70c9	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	808960		

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/22 (10-20)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) P1143.70000US05
Application Number 17/347,435-Conf. #4020	Filed June 14, 2021
For SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	
Art Unit Not Yet Assigned	Examiner Not Yet Assigned

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$220	$110	$55	$
☐ Two months (37 CFR 1.17(a)(2))	$640	$320	$160	$
☐ Three months (37 CFR 1.17(a)(3))	$1,480	$740	$370	$
☐ Four months (37 CFR 1.17(a)(4))	$2,320	$1,160	$580	$
☒ Five months (37 CFR 1.17(a)(5))	$3,160	$1,580	$790	$ 1,580.00

☒ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. ~~Form PTO-2038 is attached.~~

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 23/2825 .

☒ Payment made via EFS-Web.

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.

I am the

☐ applicant.

☐ attorney or agent of record. Registration number _____ .

☒ attorney or agent acting under 37 CFR 1.34. Registration number 48,644 .

/Andrew W. Williams/	January 28, 2022
Signature	Date
Andrew W. Williams, PhD	617.646.8000
Typed or printed name	Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of 1 forms are submitted.

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: January 28, 2022 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

9996550.1

REMARKS

Applicant respectfully requests entrance of this Preliminary Amendment to introduce a Substitute Specification (clean and marked-up copies) and Sequence Listing (submitted as a text file) to identify amino acid and/or nucleotide sequences disclosed in the specification in accordance with the requirements of 37 C.F.R §§ 1.821 through 1.825. Applicant submits that the substitute Sequence Listing includes no new matter.

Applicant also respectfully requests entrance of this Preliminary Amendment to reduce the number of claims pending in the Application, obviating excess claims fees. After entrance of the present Amendment, one (1) independent claim, twenty (20) total claims, and no multiple dependent claims will be pending in this case. Claims 1-295 have been canceled without prejudice or disclaimer. Applicant reserves the right to pursue subject matter canceled from the claims in future applications claiming priority to the present Application. New claims 296-315 have been added. Support for the new claims can be found throughout the Application and claims as originally filed. Applicant submits that no new matter is added to the Application by this Preliminary Amendment.

Applicant believes all fees due are being paid by credit card. However, if any additional fees are due, please charge them, or credit any overpayment, to our Deposit Account No. 23/2825, under Docket No. P1143.70000US05, from which the undersigned is authorized to draw.

Dated: January 28, 2022

Respectfully submitted,

By: /Andrew W. Williams/
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Registration No.: 48,644
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600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of and claims under 35 U.S.C. § 120 to U.S. Patent Application U.S.S.N. 16/089,040, filed September 27, 2018, which is a national stage filing under 35 U.S.C. § 371 of International PCT Application PCT/US2017/025621, filed March 31, 2017, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application, U.S.S.N. 62/316,555, filed on March 31, 2016; U.S. Provisional Application, U.S.S.N. 62/330,245, filed on May 2, 2016; U.S. Provisional Application, U.S.S.N. 62/372,263, filed on August 8, 2016; and U.S. Provisional Application, U.S.S.N. 62/373,807, filed on August 11, 2016, each of which is herein incorporated by reference.

SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 27, 2022, is named P114370000US05-SEQ-AZW and is 5,194 bytes in size.

SUMMARY

[0003] 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.

[0004] 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,

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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 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.

[0005] 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,

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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).

[0006] 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 has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

[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 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] 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.

[0009] Provided herein is a kit comprising of any pharmaceutical composition described herein or any pharmaceutical composition contained in a container described herein.

[0010] Provided herein are methods of making compositions, methods of immunizing with compositions, and methods of vaccinating with compositions.

INCORPORATION BY REFERENCE

[0011] 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.

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BRIEF DESCRIPTION OF THE FIGURES

[0012] 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:

[0013] FIGURE 1 illustrates a flow diagram of the general process for manufacture of a saccharide peptide conjugate vaccine.

[0014] FIGURE 2 illustrates a flow diagram of a process for cell banking of a bacterial strain.

[0015] FIGURE 3 illustrates a flow diagram of a process for producing, isolating, and purifying CRM_{197}.

[0016] FIGURE 4 illustrates a flow diagram of a fermentation process for isolating a polysaccharide from a strain of *Streptococcus pneumoniae*.

[0017] FIGURE 5 illustrates a flow diagram of a conjugation process for conjugating a polysaccharide and CRM_{197}.

[0018] FIGURE 6 illustrates a flow diagram for a process for formulating a polysaccharide polypeptide conjugate vaccine.

[0019] 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.

[0020] 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

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serotypes in the Liffey/Vax ePCV8 is predicted to cover about 23% more of IPD when administered in a vaccination regime with PCV20.

[0021] 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 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.

[0022] 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

[0023] 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.

[0024] 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".

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[0025] 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.

[0026] 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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

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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%.

[0031] 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.

[0032] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0033] 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}.

[0034] 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.

[0035] 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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[0036] 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.

[0037] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0038] The term "plurality" can mean two or more.

[0039] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

[0040] 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)*.

[0041] 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).

[0042] 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

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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

[0043] 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. 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.

[0044] 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-Pnc*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.

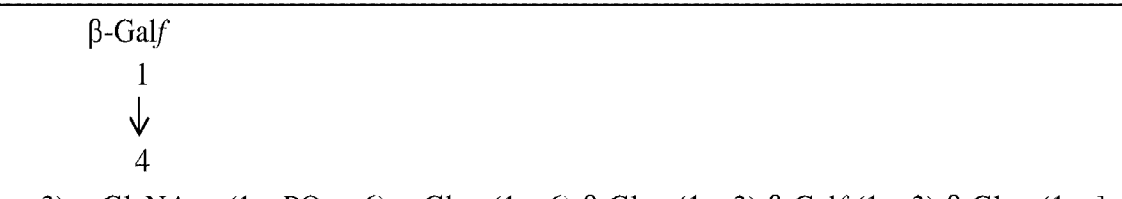
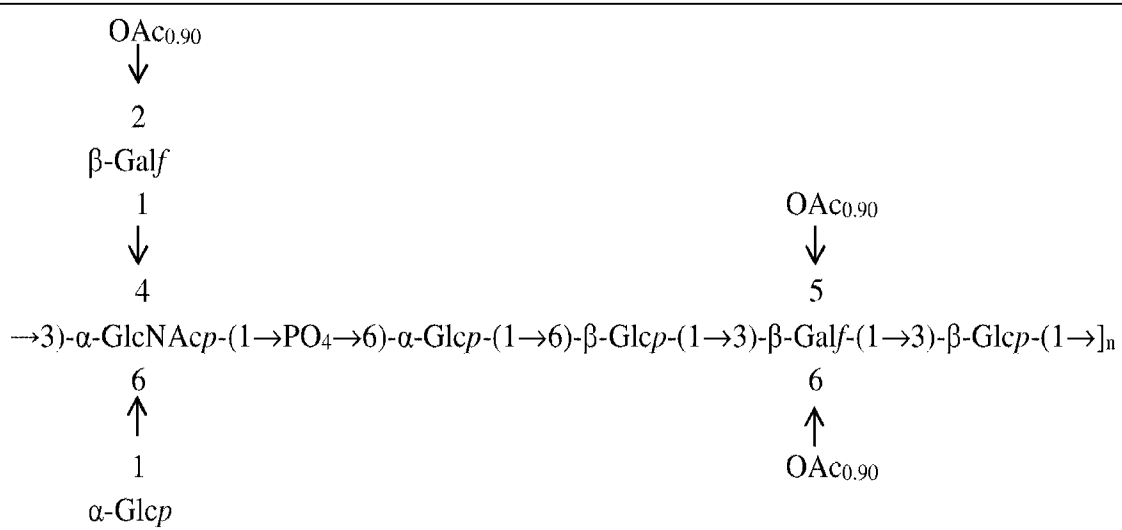
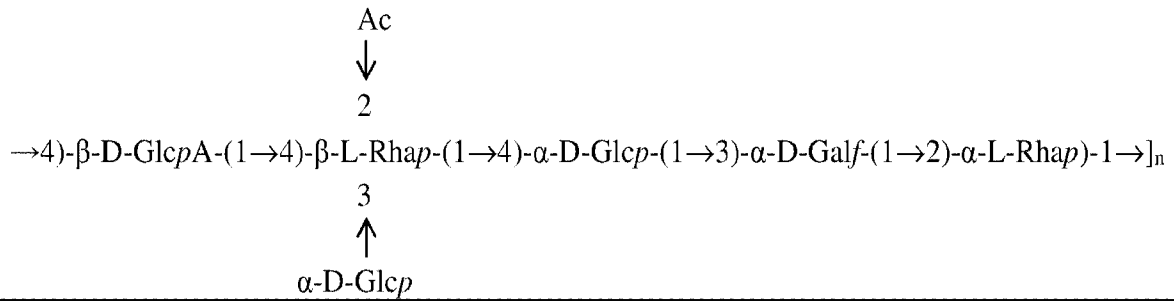
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$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

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Serotype	Polysaccharide Structure (Repeat Unit)
	β-D-Gal*p*
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-PO4$^-$-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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[0045] 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.

[0046] 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.

[0047] 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.

[0048] The saccharides or polysaccharides of the invention are depolymerized (sized) to a final range of 100-500 kDa.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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}.

[0053] 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*.

[0054] 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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[0055] 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

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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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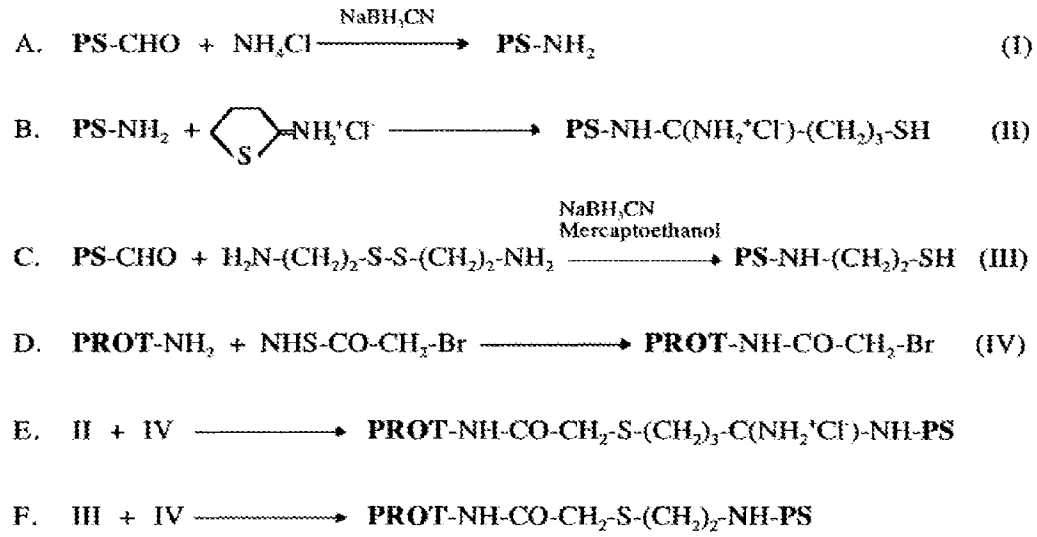
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[0063] 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.

[0064] 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.

[0065] 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

[0066] 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).

[0067] 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.

[0068] 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.

[0069] 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

[0070] 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).

[0071] 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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[0072] 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*.

[0073] 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.

[0074] 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.

[0075] 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 Palmitate, 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.

[0076] 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.

[0077] 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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[0078] 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.

[0079] 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

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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*.

[0090] 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*.

[0091] 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.

[0092] 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.

[0093] 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

[0094] 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

[0095] 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

[0096] 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.

[0097] *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.

[0098] *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

[0099] 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).

[0100] 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

[0101] 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

[0102] 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

[0103] 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.

[0104] 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

[0105] 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

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List of Polysaccharide Serotype Combinations
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 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

[0106] 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

[0107] 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

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appropriate volume of each monovalent bulk polysaccharide-CRM_{197} conjugate is added (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

[0108] 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.

[0109] 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

[0110] 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

[0111] 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

[0112] 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

[0113] 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

[0114] 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

[0115] 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

[0116] 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*

[0117] 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.

[0118] 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*

[0119] 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.

[0120] 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*

[0121] 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.

[0122] 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

[0123] 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.

[0124] 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

[0125] 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.

[0126] 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

[0127] 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.

[0128] 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

[0129] 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.

[0130] 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

[0131] 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.

[0132] 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*

[0133] 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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[0134] 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*

[0135] 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.

[0136] 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*

[0137] 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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[0138] 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.

[0139] 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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Doc Code: TRAN.LET
Document Description: Transmittal Letter

USED IN LIEU OF PTO/SB/21 (07-09)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	17/347,435-Conf. #4020
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	P1143.70000US05

ENCLOSURES (*Check all that apply*)

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [x] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [x] Reply to Missing Parts/ Incomplete Application
  - [x] Reply to Missing Parts under 37 CFR 1.52 or 1.53

- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD

Remarks

- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Sequence Listing (.txt file)

Substitute Specification (marked-up and clean versions)

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Andrew W. Williams/		
Printed name	Andrew W. Williams, PhD		
Date	January 28, 2022	Reg. No.	48,644

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Dated: January 28, 2022 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

9996548.1

FEE TRANSMITTAL

Complete if known	
Application Number	17/347,435-Conf. #4020
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Examiner Name	Not Yet Assigned
Art Unit	Not Yet Assigned
Practitioner Docket No.	P1143.70000US05

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($)2,490.00

METHOD OF PAYMENT (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

[ ] Charge fee(s) indicated below

[ ] Charge fee(s) indicated below, **except for the filing fee**

[x] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**

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[x] Credit any overpayment of fee(s)

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	320	160*	80	700	350	175	800	400	200	830.00
Design	220	110	55	160	80	40	640	320	160	
Plant	220	110	55	440	220	110	660	330	165	
Reissue	320	160	80	700	350	175	2,320	1,160	580	
Provisional	300	150	75	0	0	0	0	0	0	

* The $160 small entity status filing fee for a utility application is further reduced to $80 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	480	240	120
Multiple dependent claims	860	430	215

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
20 - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**
_____	_____

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $420 ($210 for small entity) ($105 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

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

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge): 2255 Extension for response within fifth month	1,580.00
2051 Surcharge - Late filing fee, search fee, ...	80.00

SUBMITTED BY

Signature	/Andrew W. Williams/	Registration No. (Attorney/Agent)	48,644	Telephone	617.646.8000
Name (Print/Type)	Andrew W. Williams, PhD			Date	January 28, 2022

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Application Number: 17347435 Document Date: 01/28/2022

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Electronic Acknowledgement Receipt

EFS ID:	44870061
Application Number:	17347435
International Application Number:	
Confirmation Number:	4020
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. Forrest
Customer Number:	23628
Filer:	Andrew W. Williams/Chelsea Dowling
Filer Authorized By:	Andrew W. Williams
Attorney Docket Number:	P1143.70000US05
Receipt Date:	28-JAN-2022
Filing Date:	14-JUN-2021
Time Stamp:	22:08:35
Application Type:	Utility under 35 USC 111(a)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Sequence Listing (Text File)	P114370000US05-SEQ-AZW.TXT	5194	no	-

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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.

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Sequence Listing was accepted.
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217-9197 (toll free).
Reviewer: Anjum, Durreshwar
Timestamp: [year=2022; month=1; day=31; hr=6; min=3; sec=4; ms=331; ]
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Validated By CRFValidator v 1.0.5

Application No: 17347435 Version No: 1.0

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Finished: 2022-01-28 22:09:33.634
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Total Warnings: 1
Total Errors: 0
No. of SeqIDs Defined: 1
Actual SeqID Count: 1

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<120> SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

<130> P1143.70000US05

<140> US 17/347,435
<141> 2021-06-14

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<151> 2018-09-27

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Asp Trp Lys Glu Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala Gly

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Leu Phe Phe Glu Ile Lys Ser
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
17/347,435	06/14/2021		910	P1143.70000US05	20	1

CONFIRMATION NO. 4020

UPDATED FILING RECEIPT

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

OC000000131497408

Date Mailed: 02/02/2022

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Bruce D. Forrest, South Nyack, NY;
Jack D. Love, Tampa, FL;

Applicant(s)

Pogona, LLC, South Nyack, NY;

Assignment For Published Patent Application

Pogona, LLC, South Nyack, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/089,040 09/27/2018 PAT 11058757
which is a 371 of PCT/US2017/025621 03/31/2017
which claims benefit of 62/373,807 08/11/2016
and claims benefit of 62/372,263 08/08/2016
and claims benefit of 62/330,245 05/02/2016
and claims benefit of 62/316,555 03/31/2016

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Petitioner Merck Ex-1015, 0374

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 06/24/2021

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 17/347,435**

Projected Publication Date: 05/12/2022

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor

community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Petitioner Merck Ex-1015, 0377

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 17/347,435

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	80		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	350		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	400		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*	x 240 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	830		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/347,435	06/14/2021	Bruce D. Forrest	P1143.70000US05

CONFIRMATION NO. 4020

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

PUBLICATION NOTICE

OC000000133808291

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

Publication No.US-2022-0143167-A1
Publication Date:05/12/2022

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Petitioner Merck Ex-1015, 0379

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/347,435	06/14/2021	Bruce D. Forrest	P1143.70000US05	4020

23628 7590 02/20/2024
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

EXAMINER
SHAHNAN SHAH, KHATOL S

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
02/20/2024	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Office Action Summary	Application No. 17/347,435	Applicant(s) Forrest et al.	
	Examiner KHATOL S SHAHNAN SHAH	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 1/28/2022.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 296-315 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☑ Claim(s) 296-315 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Restrictions

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Applicants' amendment of 1/28/2022 is acknowledged. Claims 1-295 have canceled. New claims 296-315 have been added.

Status of Claims

3. Claims 296-315 are pending in this application.

4. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 296-309 are drawn to a pharmaceutical composition; classified in class A61k, subclass 39/092.

II. Claims 310-314 are drawn to a method of administering a composition; classified in class A61k, subclass 2039/70.

III. Claim 315 is drawn to a method of preparing a composition; classified in class A61k, subclass 47/646.

5. The inventions are distinct, each from the other because of the following reasons: The inventions are distinct because invention I is drawn to a composition and invention II and III are drawn to distinct methods with different method steps.

Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case a different composition can be used in a method to elicit an immune response.

Inventions I and III are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2)

that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case different polysaccharides can be used by a method to produce an hyperimmunized animal.

6. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is

the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

7. The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provisions of MPEP § 821.04. **Process claims that depend from or otherwise include all the limitations of the patentable product** will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final rejection are governed by 37 CFR 1.116; amendments submitted after allowance are governed by 37 CFR 1.312. In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103, and 112. Until an elected product claim is found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowed product claim will not be rejoined. See "Guidance on Treatment of Product and Process Claims in light of In re Ochiai, In re Brouwer and 35 U.S.C. § 103(b)," 1184 O.G. 86 (March 26, 1996). Additionally, in order to retain the right to rejoinder in accordance with the above policy, Applicant is advised that the process claims should be amended during prosecution either to maintain dependency on the product claims or to otherwise include the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

8. Applicants are reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one

or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(l).

Conclusion

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KHATOL S SHAHNAN SHAH whose telephone number is (571)272-0863. The examiner can normally be reached on Mon-Tue, Thurs-Fri 12pm-8pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary B. Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Khatol S Shahnan-Shah/
Examiner, Art Unit 1645
February 9, 2024

/JANA A HINES/
Primary Examiner, Art Unit 1645

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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.

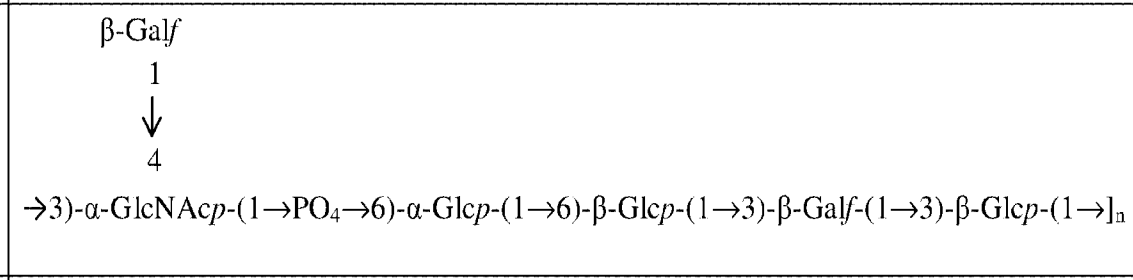
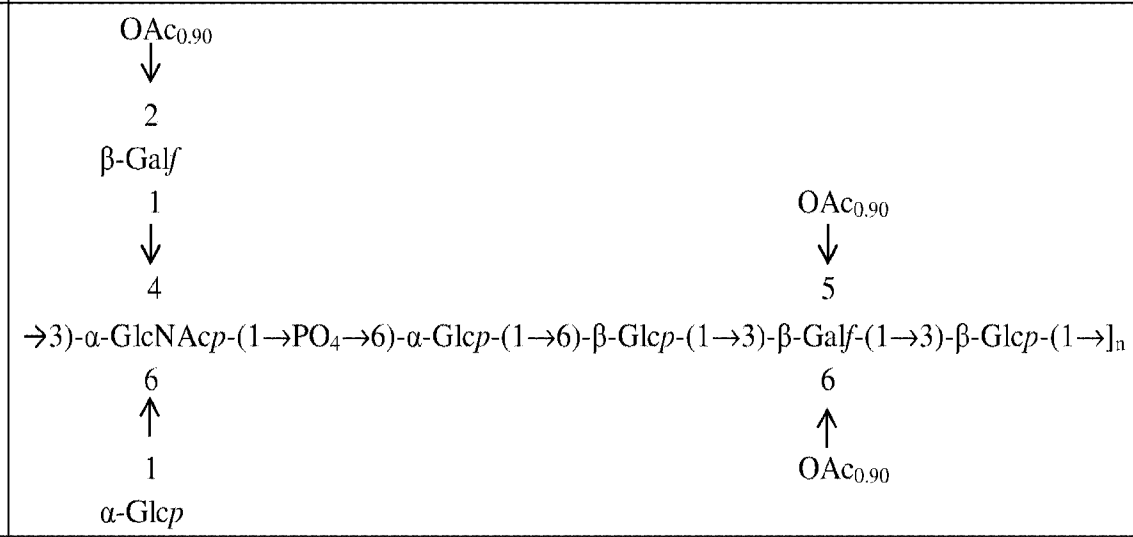
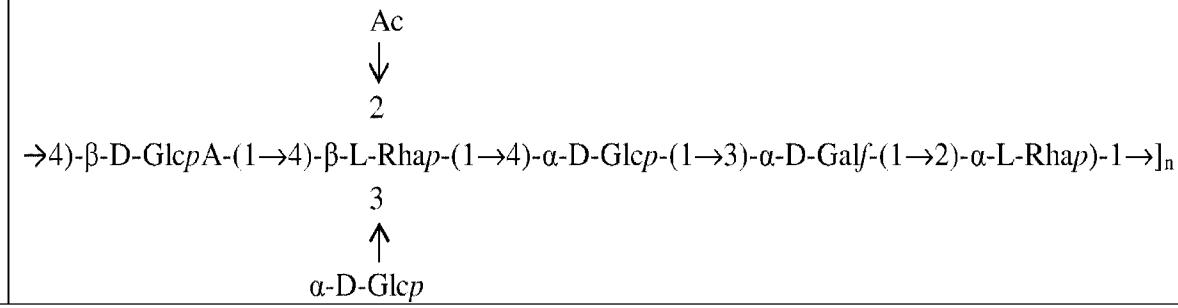
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Serotype	Polysaccharide Structure (Repeat Unit)
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 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-(l→]n 3 ↑ PO4-1-glycerol
19F	→2)-α-L-Rha-(1→5-PO4^{-}-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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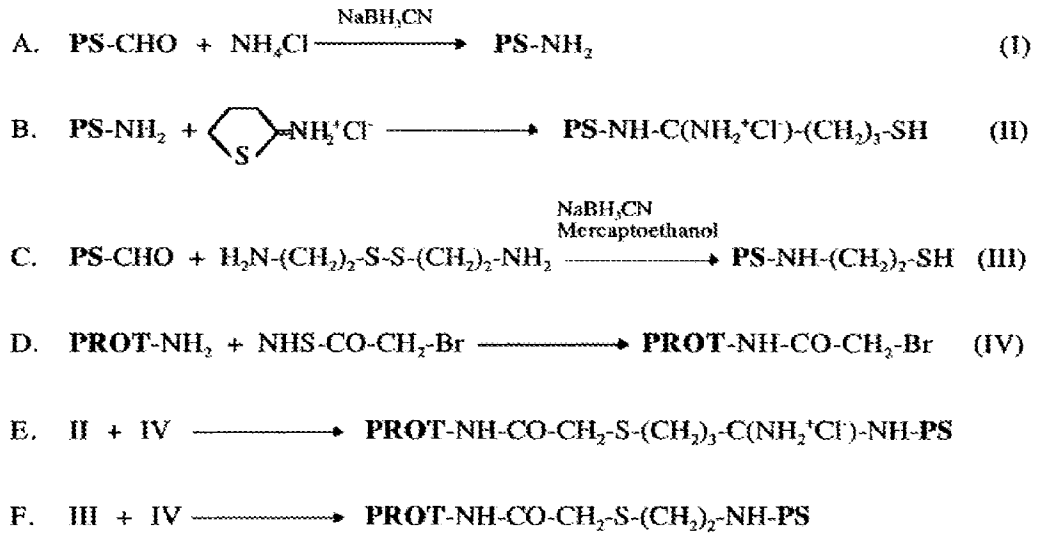
The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[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.*, Oxydcoxynucleotides 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 Palmitate, 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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