SYNTHETIC OLIGOSACCHARIDES FOR P. *AERUGINOSA* VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

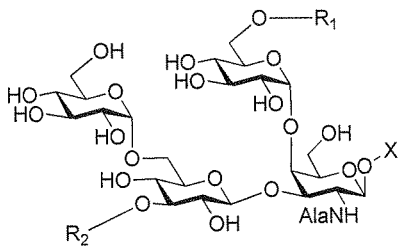
[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/842,474, filed July 3, 2013, the entire contents of which are hereby incorporated herein by reference.

FIELD OF THE INVENTION

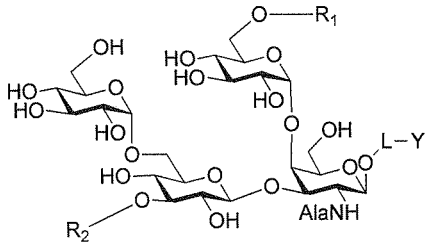
[0002] The present invention relates to immunogenic and immunoprotective compositions and methods for making and using homogenous synthetic *P. aeruginosa* lipooligosaccharide (LOS)-based oligosaccharides, conjugates, and antibodies derived therefrom.

SUMMARY

[0003] In an aspect, ~~T~~the present ~~invention~~ disclosure provides synthetic oligosaccharide **1a** or **1b**:



1a



1b

where each of R^1 and R^2 is independently H, a monosaccharide or an oligosaccharide, X is H, a linker group, or a protecting group; L is a linker and Y is a carrier. In some embodiments, R^1 and R^2 are each independently H, α-Rha-, α-Glc(1-2)-α-Rha-, β-QuiNAc(1-3)-α-Rha-, β-FucNAc(1-3)-α-Rha-, α-Rha[2,3,4-OAc]-, β-QuiNAc(1-3)-α-Rha[2,4-OAc]-, or β-FucNAc(1-3)-α-Rha[2,4,-OAc]-. In some embodiments, R^1 and R^2 are selected from the combinations in the following table:

R^1	R^2
α-Rha-	H
α–Glc(1-2)-α-Rha-	H
H	α-Rha-
H	β–QuiNAc(1-3)-α-Rha-
H	β–FucNAc(1-3)-α-Rha-
H	α-Rha[2,3,4-OAc]-
H	β-QuiNAc(1-3)-α-Rha[2,4-OAc]-
H	β-FucNAc(1-3)-α-Rha[2,4,-OAc]-

[0004] The present ~~invention~~ disclosure also includes compositions comprising an antigen **1a** and/or **1b** and a pharmaceutically acceptable vehicle. In another aspect, the present disclosure provides a composition comprising a synthetic oligosaccharide as disclosed herein and a pharmaceutically acceptable vehicle. Preferably the composition contains a single antigen or a known, defined mixture of antigens.

[0005] The ~~invention~~ present disclosure further provides vaccine compositions, including immunogenic and immunoprotective compositions, comprising antigen **1a** and/or **1b** and a pharmaceutically acceptable vehicle. In an aspect, the present disclosure provides a composition comprising an oligosaccharide as disclosed herein as a vaccine. These vaccine compositions can optionally include a pharmaceutically acceptable adjuvant. Preferably, the vaccine compositions are endotoxin-free. The vaccine compositions can be mono-, di-, tri- or tetravalent. In an aspect, the present disclosure provides a method for producing an antibody against *Pseudomonas*, the method comprising: (a) administering to a non-human subject an effective amount of at least one oligosaccharide as disclosed herein; and (b) isolating the antibody from the subject. In another aspect, the present disclosure provides a method for producing a monoclonal antibody against *Pseudomonas*, the method comprising: (a) administering to a non-human subject an effective amount of at least one oligosaccharide as disclosed herein; (b) isolating the antibody from the subject; (c) fusing an antibody producing cell from

the subject to a myeloma cell; and (d) harvesting the antibody produced from a fusion subclone. In an aspect, the present disclosure provides an antibody producing cell obtained by the method as disclosed herein. In another aspect, the present disclosure provides an antibody produced by the method as disclosed herein. In another aspect, the present disclosure provides a method of detecting the presence of *Pseudomonas* in a sample, comprising contacting the sample with an antibody against an oligosaccharide as disclosed herein. In another aspect, the present disclosure provides a kit comprising an antibody against an oligosaccharide as disclosed herein.

[0006] The ~~invention~~ present disclosure further provides a method for synthetically forming oligosaccharides **1a** and antigens **1b**.

[0007] The ~~invention~~ present disclosure further provides methods for diagnosing, treating, and preventing infections caused by *P. aeruginosa*. In an aspect, the present disclosure provides a pharmaceutical composition comprising an oligosaccharide as disclosed herein for use in inducing an immune response against *Pseudomonas* in a subject. In another aspect, the present disclosure provides use of an oligosaccharide as disclosed herein in the manufacture of a medicament for inducing an immune response against *Pseudomonas* in a subject. In another aspect, the present disclosure provides an antibody preparation against an oligosaccharide as disclosed herein. In another aspect, the present disclosure provides a pharmaceutical composition comprising an antibody preparation as disclosed herein for use in providing passive immunity against *Pseudomonas* in a subject. In another aspect, the present disclosure provides use of an antibody preparation as disclosed herein in the manufacture of a medicament for providing passive immunity against *Pseudomonas* in a subject. In another aspect, the present disclosure provides a pharmaceutical composition comprising an oligosaccharide as disclosed herein or an antibody thereto for treating or preventing a disease associated with *P. aeruginosa* infection in a subject. In another aspect, the present disclosure provides use of an oligosaccharide as disclosed herein or an antibody thereto in the manufacture of a medicament for treating or preventing a disease associated with *P. aeruginosa* infection in a subject.

DETAILED DESCRIPTION

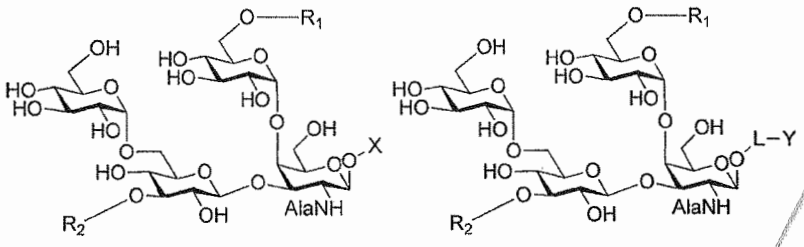
[0008] *Definitions*

[0009] In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided.

[0010] Units, prefixes, and symbols may be denoted in their SI accepted form. Numeric ranges recited herein are inclusive of the numbers defining the range and include and are supportive of each integer within the defined range. Unless

Claims

1. A synthetic oligosaccharide **1a** or **1b**



1a **1b**

where each of R^1 and R^2 is independently H, a monosaccharide or an oligosaccharide, X is H, a linker group, or a protecting group; L is a linker and Y is a carrier.

2. The oligosaccharide of claim 1, wherein each of R^1 and R^2 is independently selected from the group consisting of independently H, α-Rha-, α-Glc(1-2)-α-Rha-, β-QuiNAc(1-3)-α-Rha-, β-FucNAc(1-3)-α-Rha-, α-Rha[2,3,4-OAc]-, β-QuiNAc(1-3)-α-Rha[2,4-OAc]-, and β-FucNAc(1-3)-α-Rha[2,4,-OAc]-.

3. The oligosaccharideof claim 1, wherein R^1 and R^2 are selected from one of the combinations listed in the following table:

R^1	R^2
α-Rha-	H
α–Glc(1-2)-α-Rha-	H
H	α-Rha-
H	β–QuiNAc(1-3)-α-Rha-
H	β–FucNAc(1-3)-α-Rha-
H	α-Rha[2,3,4-OAc]-
H	β-QuiNAc(1-3)-α-Rha[2,4-OAc]-
H	β-FucNAc(1-3)-α-Rha[2,4,-OAc]-

4. The oligosaccharide of any one of claims 1 to 3, where L is an alkylenethiol linker.

5. The synthetic oligosaccharide of any one of claims 1 to 3, where Y is a carrier selected from the group consisting of proteins, peptides, lipids, polymers, dendrimers, virosomes, and virus-like particles or combination thereof.

6. The synthetic oligosaccharide of claim 5, where the carrier is a carrier protein.

7. The synthetic oligosaccharide of claim 6, where the carrier protein is selected from the group consisting of bacterial toxoids, toxins, exotoxins, and nontoxic derivatives thereof.

8. The synthetic oligosaccharide of claim 7, wherein the carrier protein is selected from the group consisting of tetanus toxoid, tetanus toxin Fragment C, diphtheria toxoid, CRM, cholera toxoid, Staphylococcus aureus exotoxins or toxoids, Escherichia coli heat labile enterotoxin, Pseudomonas aeruginosa exotoxin A, genetically detoxified variants thereof; bacterial outer membrane proteins, serotype B outer membrane protein complex (OMPC), outer membrane class 3 porin (rPorB), porins; keyhole limpet hemocyanine (KLH), hepatitis B virus core protein, thyroglobulin, albumins, and ovalbumin; pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA); purified protein derivative of tuberculin (PPD); transferrin binding proteins, peptidyl agonists of TLR-5; and derivatives and/or combinations of the above carriers.

9. The synthetic oligosaccharide of claim 8, wherein the carrier protein is selected from the group consisting of CRM 197, Neisseria meningitidis, bovine serum albumin (BSA), human serum albumin (HSA), poly(lysine:glutamic acid), flagellin of motile bacteria, and derivatives and/or combinations thereof.

10. The synthetic oligosaccharide of claim 8, wherein the carrier protein is selected from the group consisting of tetanus toxoid, CRM 197, and OMPC.

11. A pharmaceutical composition comprising a least one oligosaccharide of any one of claims 1 to 10 in an effective amount to stimulate an immune response, optionally further comprising a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of claim 11, further comprising an adjuvant.

13. The pharmaceutical composition of any one of claims 11 or 12 wherein the immune response is an antigen-specific immune response.

14. A composition comprising a synthetic oligosaccharide of any one of claims 1 to 3 and a pharmaceutically acceptable vehicle.

15. The composition of claim 14, comprising a plurality of different oligosaccharides, where each oligosaccharide is an oligosaccharide of formula **1a** or **1b.**

16. The composition of claim14, further comprising an adjuvant.

17. The composition of claim 16, where the adjuvant is selected from the group consisting of aluminum salts, RIBI, toll-like receptor agonists, AS01 AS02 AS03, AS04, AS05, CpG-oligodeoxynucleotide, MF-59, Montanide ISA-51 VG , Montanide ISA-720, Quil A, QS21, synthetic saponins, immunostimulating complexes, stearyl tyrosine, virus-like particles, reconstituted influenza virosomes, cytokines, mast cell activator compound 48/80, liposomes, muramyl dipeptides, SAF-1, and combinations thereof.

18. The composition of any of claims 16, comprising an amount of at least one oligosaccharide sufficient to confer immunity against Pseudomonas.

19. A composition comprising an oligosaccharide of any one of claims 1 to 10 as a vaccine.

20. An antibody preparation against an oligosaccharide according to any one of claims 1 to 10.

21. The antibody preparation of claim 46, where the antibody preparation comprises at least one member from the group consisting of polyclonal antibody, monoclonal antibody, mouse monoclonal IgG antibody, humanized antibody, chimeric antibody, single chain antibodies, fragment thereof, or combination thereof.

22. A method of treating a disease associated with *P. aeruginosa* infection, comprising administering effective amount for inducing an immune response against Pseudomonas of an oligosaccharide of any of claims 1 to 10 or antibody thereto.

23. A method of treating a disease associated with *P. aeruginosa* infection, comprising administering to a patient in need thereof an oligosaccharide or composition of any of claims 1 to 19.

24. The method of claim 22 or 23, wherein the patient is human.

25. A method for producing antibodies comprising:

(a) administering to a subject an effective amount of at least one oligosaccharide of any one of claims 1 to 10, for producing antibodies specific for Pseudomonas; optionally further comprising an adjuvant.

(b) isolating antibodies from the subject.

26. A method for producing monoclonal antibodies comprising:

(a) administering to a subject an effective amount of at least one oligosaccharide of any one of claims 1 to 10, for producing antibodies specific Moraxella;

(b) isolating antibodies from the subject.

(c) fusing antibody producing cells from the subject to myeloma cells, and

(d) harvesting antibodies produced from a fusion subclone.

27. The method of claim 25 and 26, wherein the subject is a rabbit.

28. The method of claim 25 and 26, wherein the subject is a human.

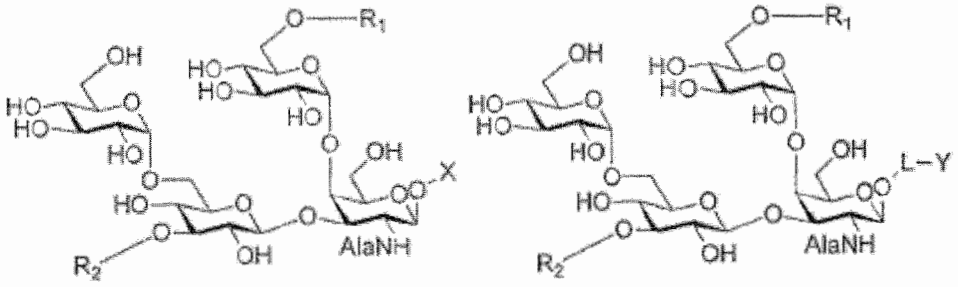
29. An antibody producing cell obtainable by performing steps (a) to (c) of claim 26.

30. An antibody obtainable by performing steps (a) to (d) of claim 26.

31. A method of diagnosing the presence of Pseudomonas in a sample, comprising contacting the sample with an antibody of claim 20, 21, 29, or 30.

Claims

1. A synthetic oligosaccharide **1a** or **1b**



1a **1b**

where each of R^1 and R^2 is independently H, a monosaccharide or an oligosaccharide, X is H, a linker group, or a protecting group; L is a linker and Y is a carrier.

2. The oligosaccharide of claim 1, which is oligosaccharide 1a.

3. The oligosaccharide of claim 1, which is oligosaccharide 1b.

4. The oligosaccharide of any of claims 1-3, wherein each of R^1 and R^2 is independently chosen from H, α-Rha-, α-Glc(1-2)-α-Rha-, β-QuiNAc(1-3)-α-Rha-, β-FucNAc(1-3)-α-Rha-, α-Rha[2,3,4- OAc]-, β-OuiNAc(1-3)-α-Rha[2,4-OAc]-, or β-FucNAc(1-3)-α-Rha[2,4,-OAc]-.

5. The oligosaccharide of any of claims 1-4, wherein R^1 and R^2 are selected from one of the combinations listed in the following table:

R^1	**R^2**
α-Rha-	H
α-Glc(1-2)-α-Rha-	H
H	α-Rha-

H	β-QuiNAc(1-3)- α-Rha-
H	β-FucNAc(1-3)- α-Rha-
H	α-Rha[2,3,4-OAc]-
H	β-QuiNAc(1-3)- α-Rha[2,4-OAc]-
H	β-FucNAc(1-3)- α-Rha[2,4,-OAc]-

6. The oligosaccharide of any of claims 1-2 or 4-5, wherein oligosaccharide 1a has formula (2a), (3a), (4a), (5a), (6a), (7a), (8a), or (9a).

7. The oligosaccharide of any of claims 1 or 3-5, wherein oligosaccharide 1b has formula (2b), (3b), (4b), (5b), (6b), (7b), (8b), or (9b).

8. The oligosaccharide of any one of claims 1, 3-5, or 7, where L is an alkylenethiol linker.

9. The oligosaccharide of any one of claims 1, 3-5, or 7-8, where Y is a carrier chosen from a protein, a peptide, a lipid, a polymer, a dendrimer, a virosome, a virus-like particle, or a combination thereof.

10. The oligosaccharide of claim 9, where the carrier is a carrier protein.

11. The oligosaccharide of claim 10, where the carrier protein is chosen from a bacterial toxoid, a toxin, an exotoxin, and a nontoxic derivative thereof.

12. The oligosaccharide of claim 11, wherein the carrier protein is chosen from tetanus toxoid, tetanus toxin Fragment C, diphtheria toxoid, CRM, cholera toxoid, a *Staphylococcus aureus* exotoxin or toxoid, *Escherichia coli* heat labile enterotoxin, *Pseudomonas aeruginosa* exotoxin A, a genetically detoxified variant thereof, a bacterial outer membrane protein, serotype B outer membrane protein complex (OMPC), outer membrane class 3 porin (rPorB), a porin, keyhole limpet hemocyanine (KLH), hepatitis B virus core protein, thyroglobulin, an

albumin, ovalbumin, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA), a purified protein derivative of tuberculin (PPD), a transferrin binding protein, a peptidyl agonist of TLR-5, or a derivative and/or a combination of the above carriers.

13. The oligosaccharide of claim 12, wherein the carrier protein is chosen from CRM 197, Neisseria meningitidis, bovine serum albumin (BSA), human serum albumin (HSA), poly(lysine:glutamic acid), flagellin of motile bacteria, or a derivative and/or a combination thereof.

14. The oligosaccharide of claim 12, wherein the carrier protein is chosen from tetanus toxoid, CRM 197, or OMPC.

15. A composition comprising a synthetic oligosaccharide of any one of claims 1-14 and a pharmaceutically acceptable vehicle.

16. The composition of claim 15, comprising a plurality of different oligosaccharides, where each oligosaccharide is an oligosaccharide of formula **1a** or **1b**.

17. The composition of claim 15 or 16, further comprising an adjuvant.

18. The composition of claim 17, where the adjuvant is chosen from an aluminum salt, RIBI, a toll-like receptor agonist, AS01, AS02, AS03, AS04, AS05, CpG-oligodeoxynucleotide, MF-59, Montanide ISA-51 VG, Montanide ISA-720, Quil A, QS21, synthetic saponins, an immunostimulating complex, stearyl tyrosine, a virus-like particle, a reconstituted influenza virosome, a cytokine, mast cell activator compound 48/80, a liposome, a muramyl dipeptide, SAF-1, or a combination thereof.

19. The composition of any of claims 15-18, comprising an amount of at least one oligosaccharide sufficient to confer immunity against *Pseudomonas*.

20. A composition comprising an oligosaccharide of any one of claims 1-14 as a vaccine.

21. A pharmaceutical composition comprising an oligosaccharide of any of claims 1-14 for use in inducing an immune response against *Pseudomonas* in a subject.

22. The pharmaceutical composition of claim 21, further comprising an adjuvant.

23. The pharmaceutical composition of claim 21 or 22, wherein the immune response is an antigen-specific immune response.

24. Use of an oligosaccharide of any of claims 1-14 in the manufacture of a medicament for inducing an immune response against *Pseudomonas* in a subject.

25. An antibody preparation against an oligosaccharide according to any one of claims 1-14.

26. The antibody preparation of claim 25, where the antibody preparation comprises at least one member chosen from a polyclonal antibody, a monoclonal antibody, a mouse monoclonal IgG antibody, a humanized antibody, a chimeric antibody, a single chain antibodies, a fragment thereof, or a combination thereof.

27. A pharmaceutical composition comprising an antibody preparation of claim 25 or 26 for use in providing passive immunity against *Pseudomonas* in a subject.

28. Use of an antibody preparation of claim 25 or 26 in the manufacture of a medicament for providing passive immunity against *Pseudomonas* in a subject.

29. A pharmaceutical composition comprising an oligosaccharide of any of claims 1-14 or an antibody thereto for treating or preventing a disease associated with *P. aeruginosa* infection in a subject.

30. Use of an oligosaccharide of any of claims 1-14 or an antibody thereto in the manufacture of a medicament for treating or preventing a disease associated with *P. aeruginosa* infection in a subject.

31. The use of claim 28 or 30, wherein the subject is human.

32. A method for producing an antibody against *Pseudomonas*, the method comprising:

(a) administering to a non-human subject an effective amount of at least one oligosaccharide of any one of claims 1-14; and

(b) isolating the antibody from the subject.

33. A method for producing a monoclonal antibody against *Pseudomonas*, the method comprising:

(a) administering to a non-human subject an effective amount of at least one oligosaccharide of any one of claims 1-14;

(b) isolating the antibody from the subject;

(c) fusing an antibody producing cell from the subject to a myeloma cell; and

(d) harvesting the antibody produced from a fusion subclone.

34. The method of claim 32 or 33, wherein the subject is a rabbit.

35. The method of any of claims 32-34, further comprising administering to the subject an adjuvant.

36. An antibody producing cell obtained by the method of any of claims 33-35.

37. An antibody produced by the method of any of claims 32-35.

38. A method of detecting the presence of *Pseudomonas* in a sample, comprising contacting the sample with an antibody against an oligosaccharide of any of claims 1-14.

39. A kit comprising an antibody against an oligosaccharide of any of claims 1-14.