[0009] These oligosaccharide-linker-NH-contaminants are undesirable especially those having a molecular weight of about 100,000 or less.

[0010] In addition, tetanus toxoid is prone to oligomerization such that the toxoid can exist in monomer, dimeric, trimeric and higher oligomeric form (e.g., 4-10 toxoid units). At increasingly higher oligomerization, the number of oligosaccharides capable of binding to the toxoid on a per monomer basis decreases as the available surface area on a per monomer basis decreases due oligomerization. Accordingly, oligomers of the toxoid such as trimeric and higher are less desirable. While tedious purification processes can provide monomeric tetanus toxoid, these processes are complicated by the fact that the monomers will tend to again oligomerize overtime.

SUMMARY

[0011] In one example, this disclosure provides for a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 10 and preferably from about 10 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligomer comprises from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than 3 percent of detectable impurities each having a molecular weight of less than 100,000;

further wherein said composition comprises monomeric and dimeric toxoid with less than 10 percent of detectable higher oligomers; and

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

[0011a] In one aspect, the disclosure provides a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 10 and preferably from about 10 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligomer comprises from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than about 3 percent of detectable impurities having a molecular weight of less than about 100,000;

further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid in the vaccine while not inducing denaturation.

[0012] In one example, this disclosure provides for a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 25 and preferably from about 30 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligosaccharide groups comprise from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than 3 weight percent of detectable impurities having a molecular weight of less than 50,000;

further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers, and

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

[0012a] In one aspect, the disclosure provides a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 25 and preferably from about 30 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligosaccharide groups comprise from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than 3 percent of detectable impurities having a molecular weight of less than 50,000;

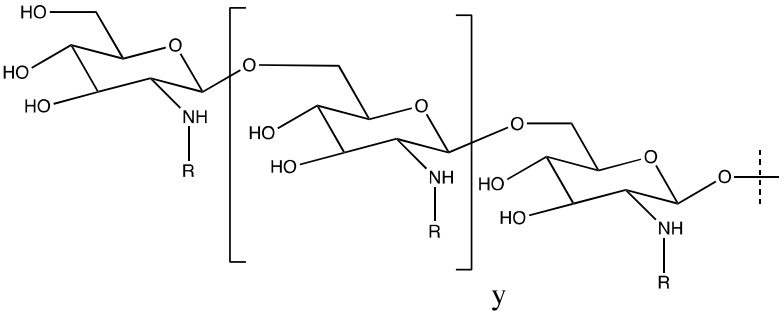
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

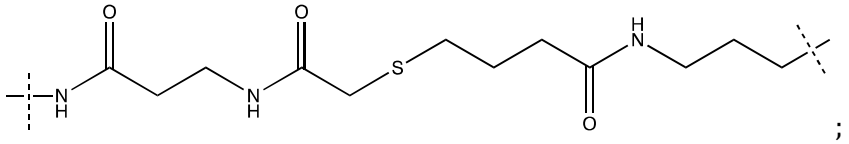
[0013] In one example, this disclosure is directed to vaccine compositions comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

where A comprises from 3 to 12 repeating β-(1→6)-glucosamine units or mixtures thereof having the formula:



B is of the formula:



where the left side of the formula is attached to C and the right side is attached to A; and C is tetanus toxoid;

x is an integer from about 10 to about 40;

y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl,

wherein said composition comprises less than 3 percent of detectable impurities having a molecular weight of about 100,000 or less;

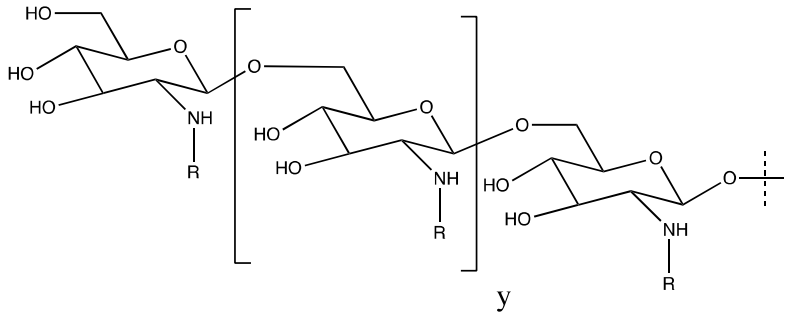
further wherein said composition comprises monomeric and dimeric toxoid with less than about 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

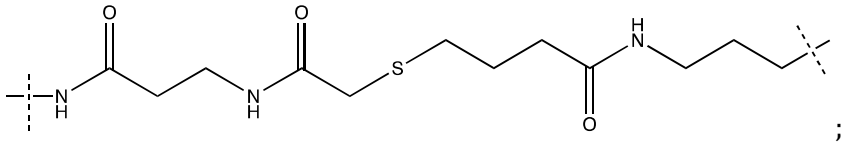
[0013a] In one aspect, the disclosure provides a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

where A comprises from 3 to 12 repeating β-(1→6)-glucosamine units or mixtures thereof having the formula:



B is of the formula:



;

where the left side of the formula is attached to C and the right side is attached to A;

and C is tetanus toxoid;

x is an integer from about 10 to about 40;

y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl,

wherein said composition comprises less than 3 percent of detectable impurities having a molecular weight of about 100,000 or less wherein said weight percent is based on the weight of vaccine compound, and

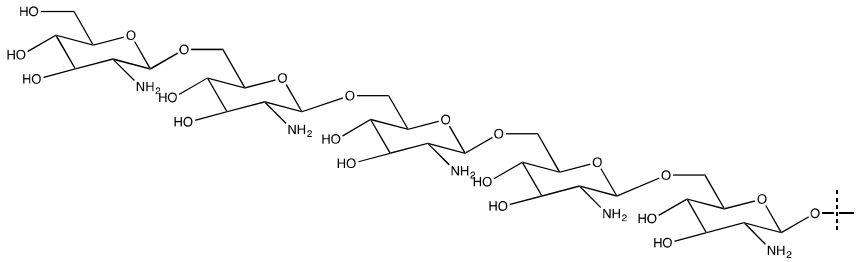
further wherein said composition comprises monomeric and dimeric toxoid with less than about 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

[0014] In one example of the vaccine composition describe above, the vaccine compound employed therein is represented by formula II:

$$(A'\text{-}B)_x\text{-}C \quad II$$

where A′ is a penta-β-(1→6)-glucosamine (carbohydrate ligand) group of the formula:

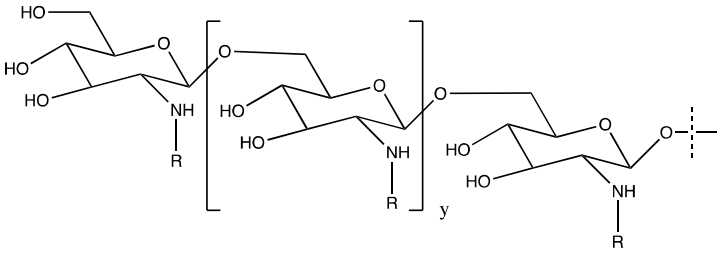


and B, C and *x* are as defined above.

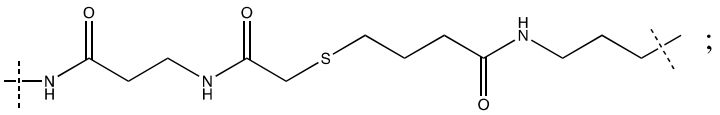
[0014a] In one aspect, the disclosure provides a composition comprising a pharmaceutically acceptable excipient and an effective amount of a conjugate of formula I:

$$(A\text{-}B)_x\text{-}C \qquad I$$

where A comprises from 3 to 12 repeating β-(1→6)-glucosamine units or mixtures thereof having the formula:



B is of the formula:



wherein each unit (A-B)- of $(A\text{-}B)_x$ is attached to C via the amide nitrogen of B indicated at the vertical dashed line of B; and

C is tetanus toxoid;

x is an integer from at least about 10 to about 40;

y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl;

wherein said composition comprises less than 3 weight percent of detectable impurities having a molecular weight of less than 50,000;

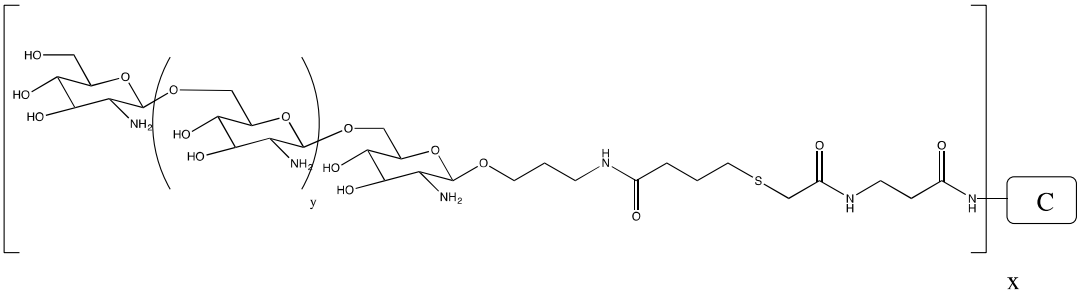
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers.

[0014b] In another aspect, the disclosure provides use of a therapeutically effective amount of the composition disclosed herein in the manufacture of a medicament for preventing or treating a microbial infection in a subject in need thereof.

[0015] In one example, the vaccines of this disclosure provide for effective immunity to a patient from microbes comprising polymeric N-acetyl-β-(1→6)-glucosamine groups in their cell wall wherein up to about 20 percent of said N-acetyl groups in the polymer are deacetylated.

[0016] In one example, this disclosure provides for a method to impart effective immunity to a patient from microbes comprising polymeric N-acetyl-β-(1→6)-glucosamine groups in their cell wall wherein up to about 20 percent of said N-acetyl groups in the polymer are deacetylated which method comprises administering a pharmaceutical composition as described herein to said patient.

[0017] Representative vaccine compounds of this disclosure are set forth in the table below:



Example	Y	C	Percent N-acetylated	*x*
A	2	Tetanus toxoid	0%	10
B	3	Tetanus toxoid	0%	15
C	6	Tetanus toxoid	12.5% (1 of 8)	20

D	10	Tetanus toxoid	25% (3 of 12)	10
E	3	Tetanus toxoid	20% (1 of 5)	20
F	4	Tetanus toxoid	33% (2 of 6)	30
G	3	Tetanus toxoid	20% (2 of 5)	35
H	3	Tetanus toxoid	0%	40

[0018] In one example, the compositions of this disclosure comprise no more about 0.5 weight percent of oligosaccharide-linked contaminants having a particle size of less than 1 micron wherein said weight percent is based on the weight of vaccine compound.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 illustrates the ^{1}H NMR for compound 17 (as described below).

[0020] Figure 2 illustrates the ^{13}C NMR for compound 17.

[0021] Figure 3 provides a HPLC trace of the conversion of the disulfide, compound 16, to two equivalents of the monosulfide, compounds 17.

DETAILED DESCRIPTION OF THE INVENTION

[0022] This invention provides for pharmaceutical compositions comprising oligosaccharide β-(1→6)-glucosamine groups.

[0023] The vaccine compositions described herein provide effective immunity to a patient against microbial infections wherein said microbe comprises oligomeric N-acetyl-β-(1→6)-glucosamine structures in its cell walls wherein up to about 20 percent of said N-acetyl groups in the polymer are deacetylated.

[0024] Prior to describing this invention in more detail, the following terms will first be defined. If a term used herein is not defined, it has its generally accepted scientific or medical meaning.

[0025] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. 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.

WHAT IS CLAIMED IS:

1. A vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 10 and preferably from about 10 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligomer comprises from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than about 3 percent of detectable impurities having a molecular weight of less than about 100,000;

further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid in the vaccine while not inducing denaturation.

2. A vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises at least 25 and preferably from about 30 to about 40 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligosaccharide groups comprise from 3 to 12 repeating β-(1→6)-glucosamine units provided that less than about 40 number percent of the total number of such units are N-acetylated

wherein said vaccine composition comprises less than 3 percent of detectable impurities having a molecular weight of less than 50,000;

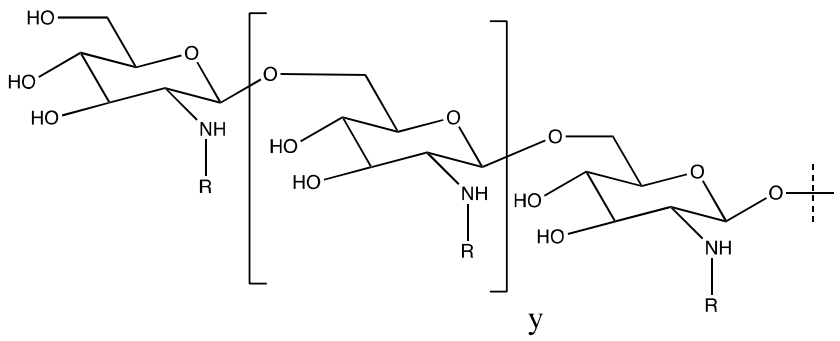
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

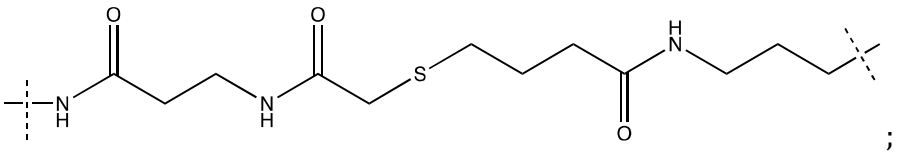
3. A vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

$$(A\text{-}B)_x\text{-}C \quad I$$

where A comprises from 3 to 12 repeating β-(1→6)-glucosamine units or mixtures thereof having the formula:



B is of the formula:



where the left side of the formula is attached to C and the right side is attached to A; and C is tetanus toxoid;

x is an integer from about 10 to about 40;

y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl,

wherein said composition comprises less than 3 percent of detectable impurities having a molecular weight of about 100,000 or less wherein said weight percent is based on the weight of vaccine compound, and

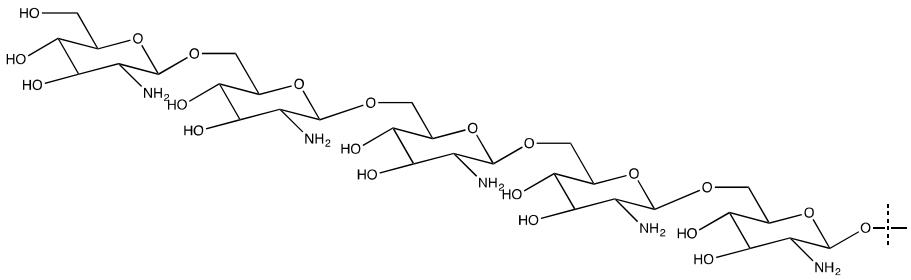
further wherein said composition comprises monomeric and dimeric toxoid with less than about 5 percent of detectable higher oligomers,

still further wherein said composition is maintained at a temperature sufficient to inhibit oligomerization of the toxoid while not inducing denaturation.

4. The vaccine composition according to claim 3, wherein the compound of formula I is represented by formula II:

$$(A'\text{-}B)_x\text{-}C \qquad II$$

where A′ is a penta-β-(1→6)-glucosamine (carbohydrate ligand) group of the formula:

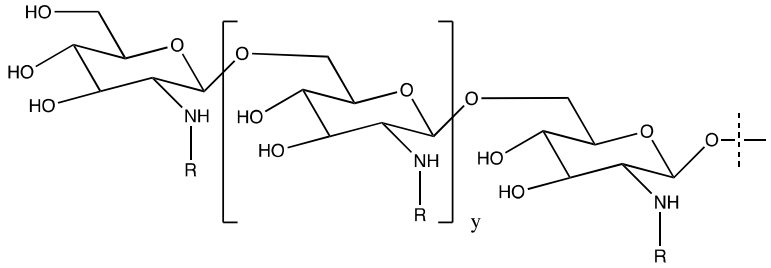


and B, C and *x* are as defined above.

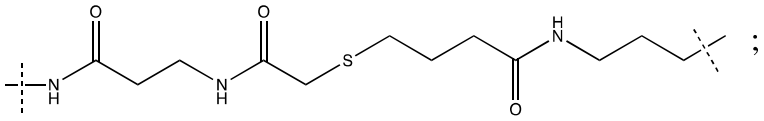
5. A composition comprising a pharmaceutically acceptable excipient and an effective amount of a conjugate of formula I:

$$(A\text{-}B)_x\text{-}C \qquad I$$

where A comprises from 3 to 12 repeating β-(1→6)-glucosamine units or mixtures thereof having the formula:



B is of the formula:



wherein each unit (A-B)- of $(A\text{-}B)_x$ is attached to C via the amide nitrogen of B indicated at the vertical dashed line of B; and

C is tetanus toxoid;

x is an integer from at least about 10 to about 40;

y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl;

wherein said composition comprises less than 3 weight percent of detectable impurities having a molecular weight of less than 50,000;

further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers.

6. The composition of claim 5, wherein R is hydrogen.

7. The composition of claim 6, wherein *y* is 3.

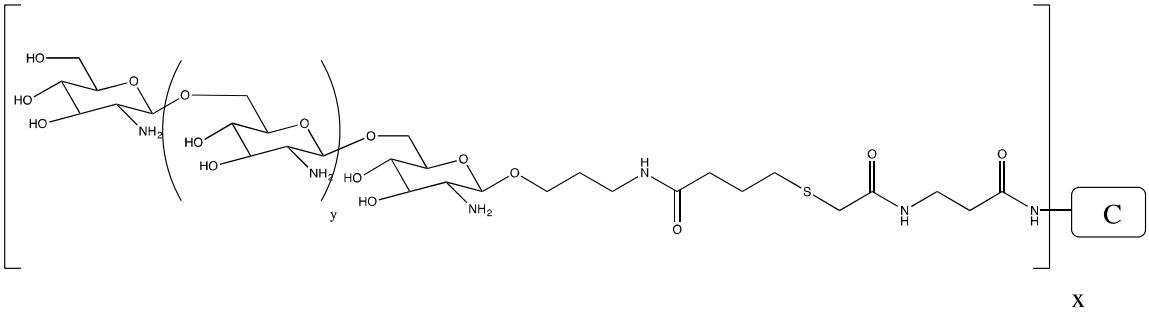
8. The composition of claim 7, wherein x is at least 25.

9. The composition of claim 5, wherein said composition is maintained at a temperature to inhibit oligomerization of the toxoid.

10. The composition of claim 9, wherein the composition is maintained at a temperature to not induce denaturation.

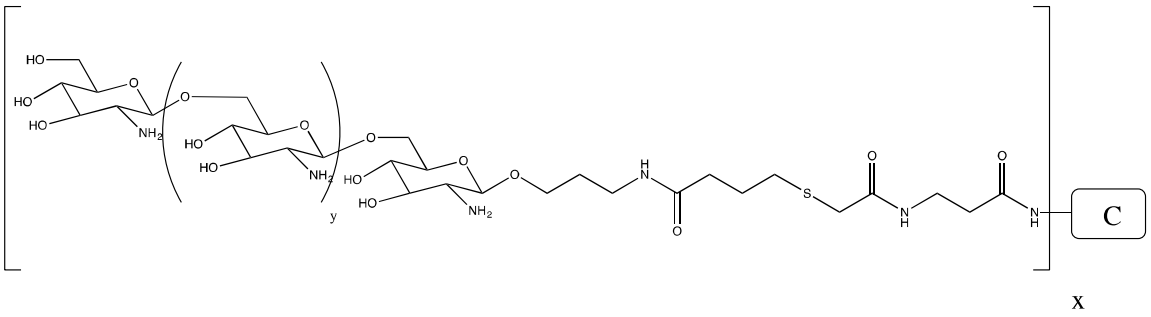
11. The composition of claim 5, wherein said composition comprises no more than about 0.5 weight percent of oligosaccharide-linked contaminants having a particle size of less than 1 micron wherein said weight percent is based on the weight of vaccine compound.

12. The composition of claim 5, wherein said conjugate is selected from:



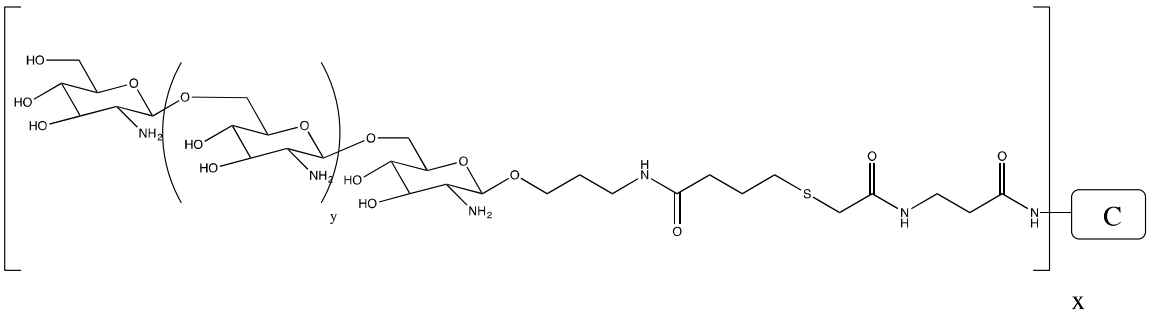
where *x* is 10, *y* is 2, C is tetanus toxoid.

13. The composition of claim 5, wherein said conjugate is selected from:



where *x* is 15, *y* is 3, C is tetanus toxoid.

14. The composition of claim 5, wherein said conjugate is selected from:



where *x* is 40, *y* is 3, C is tetanus toxoid.

15. Use of a therapeutically effective amount of the composition of any one of claims 1-14 in the manufacture of a medicament for preventing or treating a microbial infection in a subject in need thereof.

16. The use of claim 15, wherein the medicament is to be administered intramuscularly.

17. The use of claim 15 or 16, wherein one or more antibiotics is to be further administered.

18. The use of claim 15 or 16, wherein an adjuvant that potentiates the immune response to the antigen in the subject is to be further administered.

19. The use of any one of claims 15-18, wherein the microbial infection is caused by a microbe that possesses PNAG oligosaccharide β-(1→6)-glycosamide structures in its cell wall.

20. The use of any one of claims 15-19, wherein the subject is immunocompetent.