[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 from 31 to 39 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligomer comprises 5 repeating β-(1→6)-glucosamine units provided that less than 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 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 from 31 to 39 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligosaccharide groups comprise 5 repeating β-(1→6)-glucosamine units provided that less than 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;

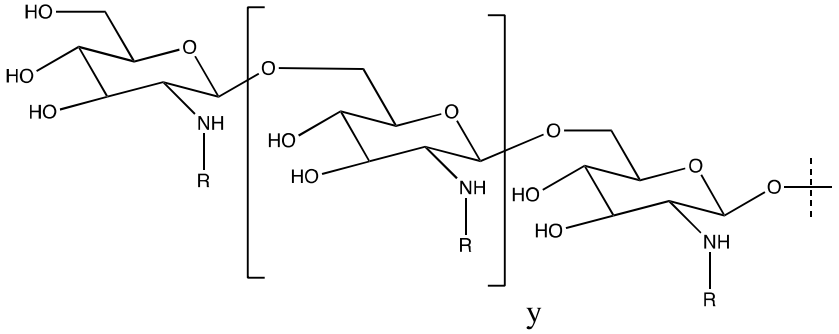
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

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

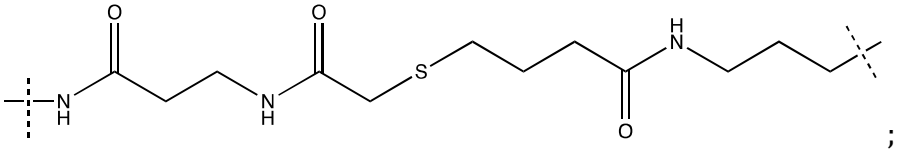
[0013] In one example, this disclosure is directed to vaccine compositions comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

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

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;

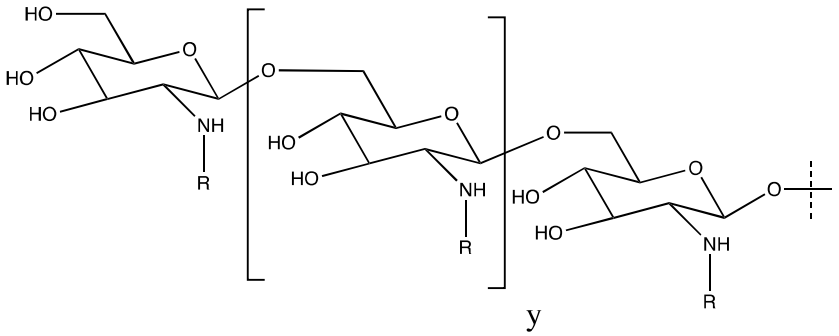
further wherein said composition comprises monomeric and dimeric toxoid with less than about 5 percent of detectable higher oligomers,

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

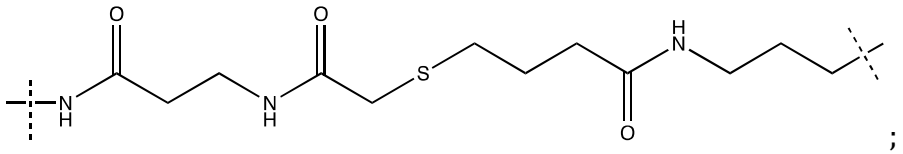
[0013a] In one aspect, the disclosure provides a vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

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

where A comprises 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 31 to 39;

y is 3; 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 100,000 or less wherein said weight percent is based on the weight of vaccine compound, and

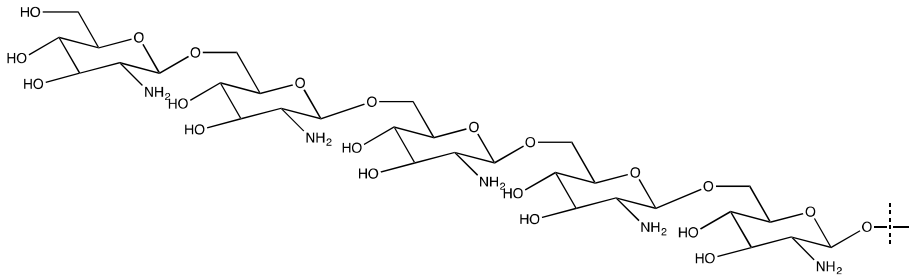
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

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:

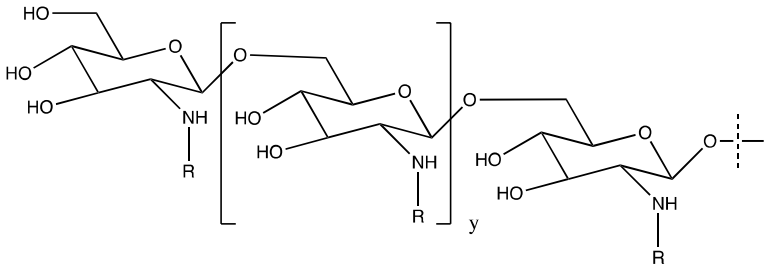


and B, C and *x* are as defined above.

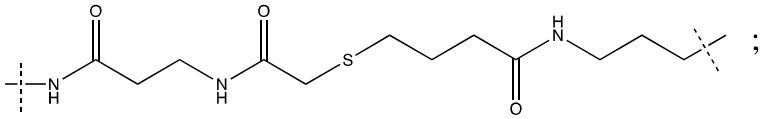
[0014a] In one aspect, the disclosure provides a composition comprising a pharmaceutically acceptable excipient and an effective amount of a conjugate of formula I:

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

where A comprises 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 31 to 39;

y is 3; 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.

WHAT IS CLAIMED IS:

1. A vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine that comprises from 31 to 39 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligomer comprises 5 repeating β-(1→6)-glucosamine units provided that less than 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 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 from 31 to 39 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker said oligosaccharide groups comprise 5 repeating β-(1→6)-glucosamine units provided that less than 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;

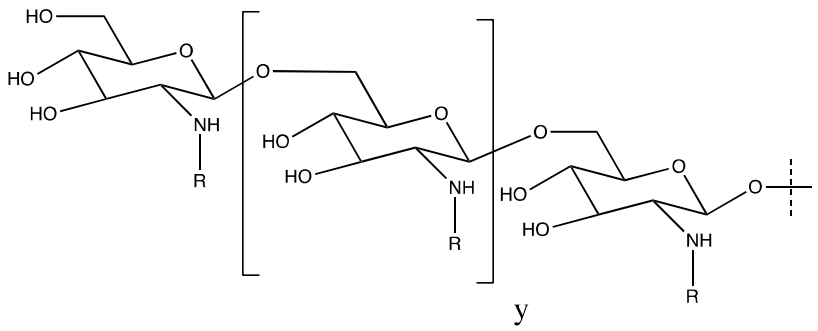
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

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

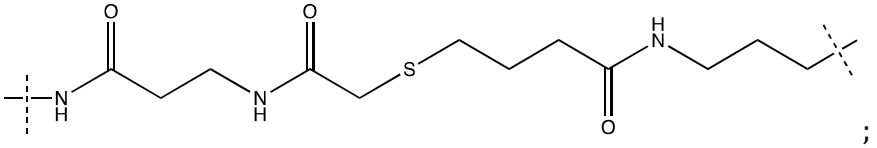
3. A vaccine composition comprising a pharmaceutically acceptable excipient and an effective amount of a vaccine compound of formula I:

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

where A comprises 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 31 to 39;

y is 3; 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 100,000 or less wherein said weight percent is based on the weight of vaccine compound, and

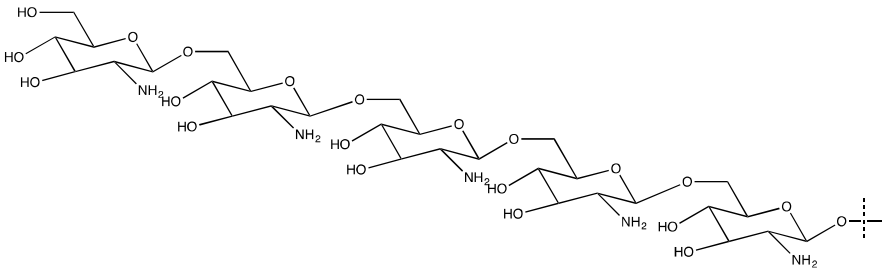
further wherein said composition comprises monomeric and dimeric toxoid with less than 5 percent of detectable higher oligomers,

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:

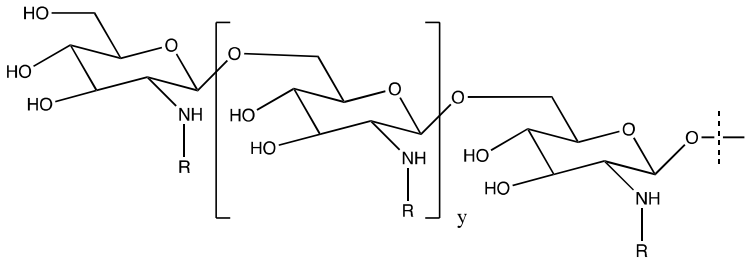


and B, C and *x* are as defined above.

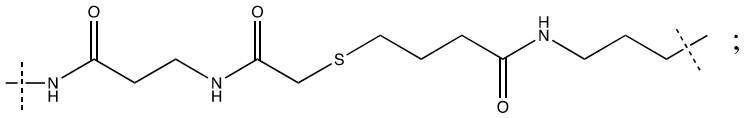
5. A composition comprising a pharmaceutically acceptable excipient and an effective amount of a conjugate of formula I:

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

where A comprises 5 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 31 to 39;

y is 3; 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.

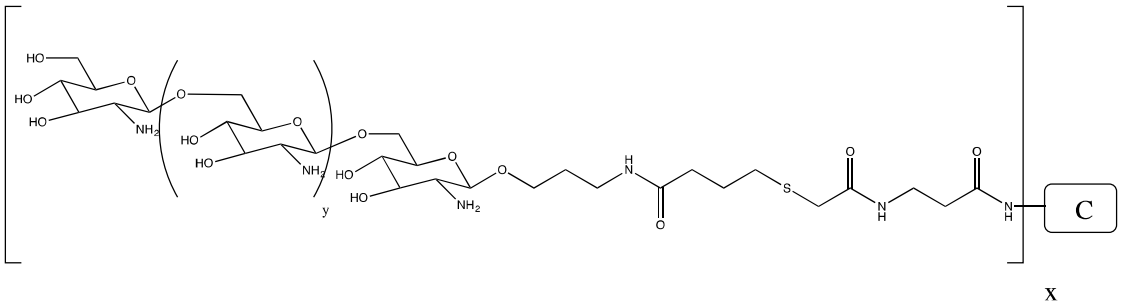
6. The composition of claim 5, wherein R is hydrogen.

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

8. The composition of claim 7, wherein the composition is maintained at a temperature to not induce denaturation.

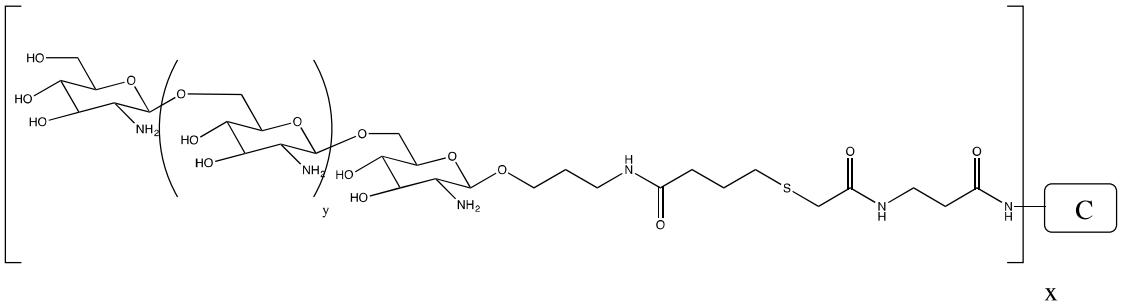
9. The composition of claim 5, wherein said composition comprises no more than 0.5 weight percent of oligosaccharide-linked contaminants having a particle size of less than 1 micron wherein said weight percent is based on the weight of vaccine compound.

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



where *x* is 31, *y* is 3, C is tetanus toxoid.

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



where *x* is 39, *y* is 3, C is tetanus toxoid.

12. Use of a therapeutically effective amount of the composition of any one of claims 1-11 in the manufacture of a medicament for preventing or treating a microbial infection in a subject in need thereof.

13. The use of claim 12, wherein the medicament is to be administered intramuscularly.

14. The use of claim 12 or 13, wherein one or more antibiotics is to be further administered.

15. The use of claim 12 or 13, wherein an adjuvant that potentiates the immune response to the antigen in the subject is to be further administered.

16. The use of any one of claims 12-15, wherein the microbial infection is caused by a microbe that possesses PNAG oligosaccharide β-(1→6)-glycosamide structures in its cell wall.

17. The use of any one of claims 12-16, wherein the subject is immunocompetent.