from manufacturer to manufacturer. Second, the manufacturing process for tetanus toxoid also results in low molecular weight contamination in the tetanus toxoid composition. These contaminants include low molecular weight reactive amino functionalities that compete with the toxoid for oligosaccharide coupling.

[0006] The art has previously disclosed antimicrobial vaccines comprising penta-β-(1→6)-glucosamine groups linked to the tetanus toxoid where the loading factor for attachment of these penta-β-(1→6)-glucosamine groups ranges from as low as 12 and up to 20 - Gening, et al., Infect. Immun., 78(2):764-772 (2010). However, this loading factor is less than desirable and apparently is based on underlying synthetic problems associated with the toxoid and coupling chemistry.

[0007] Accordingly, it would be desirable to provide for a higher level of loading onto tetanus toxoid.

SUMMARY

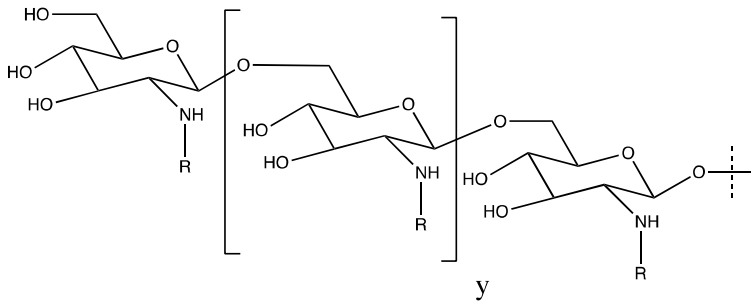
[0008] This disclosure is directed to the discovery that vaccine compounds with loading levels of at least 25 and preferably from about 31 to 39 oligomeric β-(1→6)-glucosamine–linked groups onto tetanus toxoid having from at least 25 and preferably 31 reactive amino functionalities can be achieved provided that the toxoid component in the vaccine compounds comprises at least 85 percent of the toxoid in monomeric form. In one example, the toxoid component in the vaccine compounds comprises at least 90 percent of the toxoid in monomeric form, or any subvalue or subrange there between. In some examples, the toxoid includes at least 90 percent to the 99.9 percent of the toxoid in monomeric form and preferably at least 95 percent to 99.9 percent of the toxoid in monomeric form, or any subvalue or subrange there between. In one example, the amount of low molecular weight reactive amino compounds is no more than 3 weight percent relative to the weight of toxoid present. In another example, the amount of low molecular weight amino compounds in the composition is less than 2 weight percent and preferably less than 1 weight percent based on the weight of the toxoid present and even more preferably less than 0.5 weight percent based on the weight of the toxoid present. In another preferred example, the amount of monomer is over 99 area percent, for example, based on HPLC.

[0009] Accordingly, in one example, this disclosure provides for a vaccine composition that comprises at least 25 and preferably from about 31 to about 39 oligomeric-β-(1→6)-glucosamine groups linked units onto a tetanus toxoid carrier via a linker wherein the 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 and further wherein said tetanus toxoid comprises at least 25 and preferably at least 31 reactive amino functionalities and at least 85 percent of the toxoid components are in monomeric form, or in some examples, at least 90%. Such vaccine compositions 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.

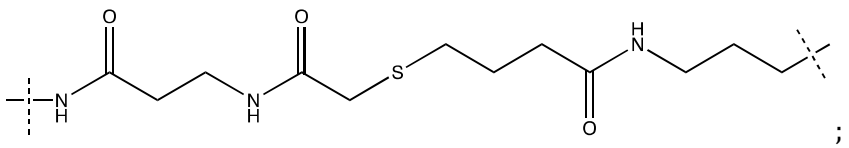
[0010] In one aspect, the present disclosure refers to a compound represented by formula I:

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

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 having at least 31 reactive amino functionalities;

x is an integer from about 31 to about 39;

y is an integer from 1 to 10; and

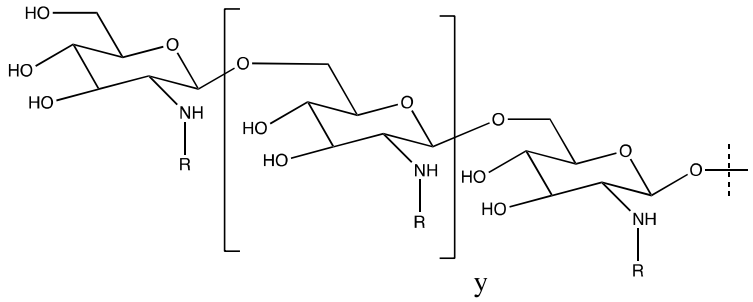
R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl

wherein said tetanus toxoid comprises at least 31 reactive amino groups and at least 90 percent by number of the toxoid is in monomeric form.

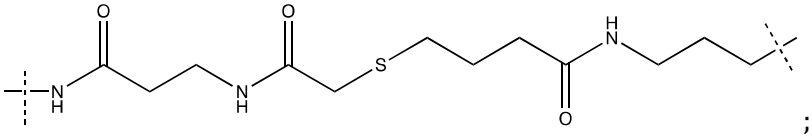
[0011] In one example, this disclosure provides for a vaccine composition that is useful against microbes which comprise oligomeric N-acetyl-β-(1→6)-glucosamine structures in their cell wall wherein said vaccine composition comprises a pharmaceutically acceptable carrier and an effective amount of a vaccine represented by 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 having at least 31 reactive amino functionalities;

x is an integer from about 31 to about 39;

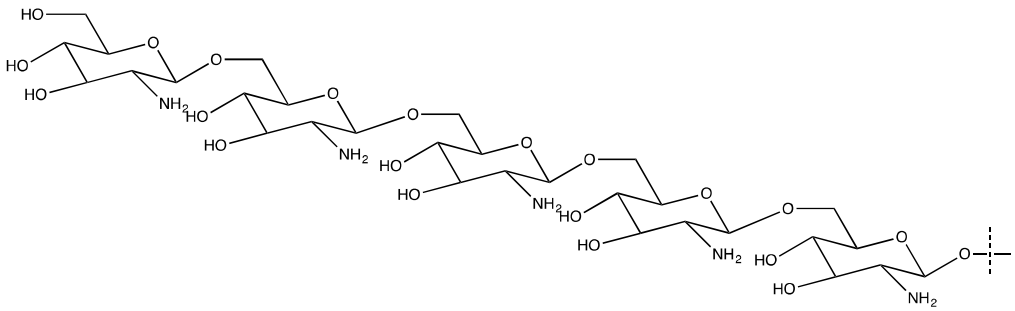
y is an integer from 1 to 10; and

R is hydrogen or acetyl provided that no more than 40% of the R groups are acetyl wherein said tetanus toxoid comprises at least 31 reactive amino groups and at least 90 percent by number of the toxoid is in monomeric form. Such vaccine compositions 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.

[0012] In one example of formula I above, there is provided a compound of formula II:

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

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

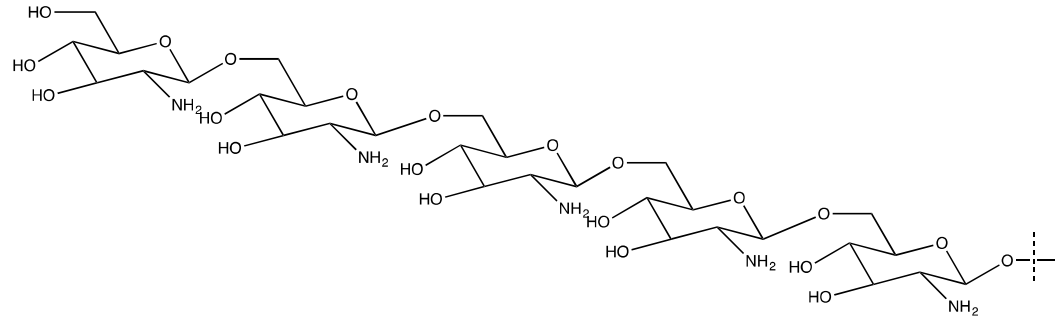


and B, C and *x* are as defined above, provided that at least 85 percent by number of the toxoid is in monomeric form, or in some examples, at least 90%.

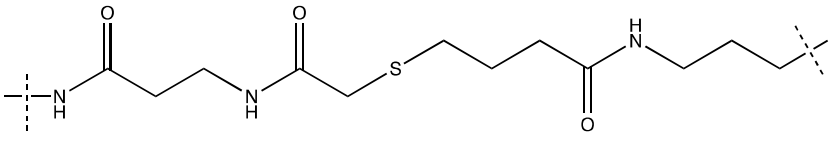
[0012a] In another aspect, the present disclosure refers to a compound represented by formula II:

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

wherein A' is



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 having at least 31 reactive amino functionalities;

x is an integer from about 31 to about 39;

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 tetanus toxoid comprises at least 31 reactive amino groups and at least 85 percent by number of the toxoid is in monomeric form.

[0012b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a pharmaceutically acceptable diluent and an effective amount of the compound disclosed herein.

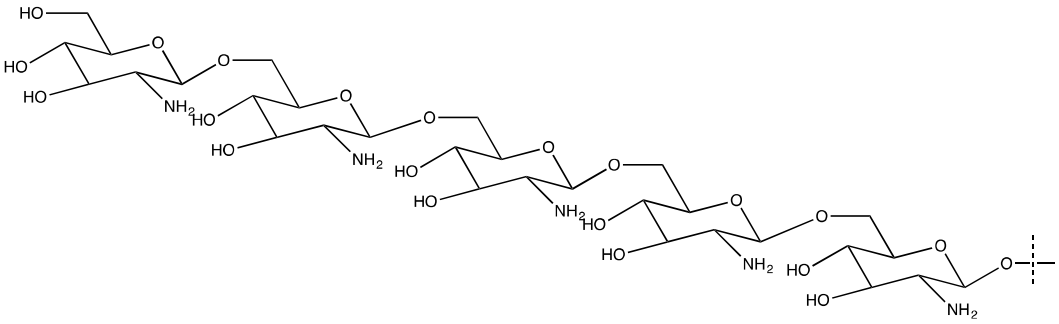
[0012c] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a pharmaceutically acceptable diluent and an effective amount of the compound disclosed herein wherein said composition comprises no more than 3 weight percent of low molecular weight amino compounds.

[0013] In one example, this disclosure provides for a vaccine composition against microbes comprising oligomeric N-acetyl-β-(1→6)-glucosamine

structures in their cell wall wherein said vaccine composition comprises a pharmaceutically acceptable carrier and an effective amount of a vaccine represented by formula II $(A'\text{-}B)_x\text{-}C$

II

where A′ has the formula



and B, C and *x* are as defined above, provided that at least 85 percent by number of the toxoid components are in monomeric form, or in some examples, at least 90%.

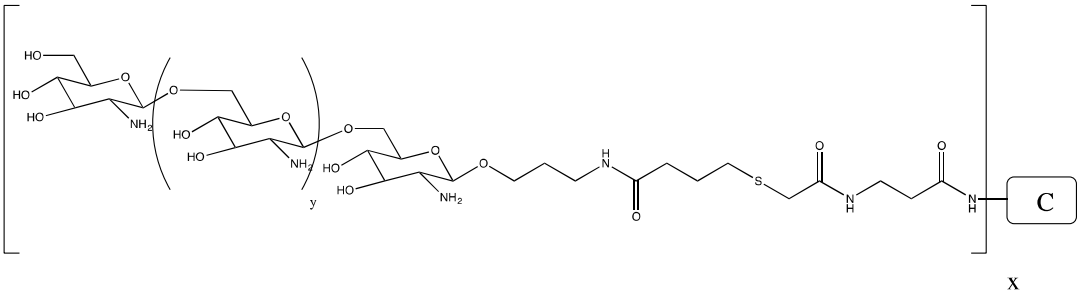
[0014] In one example, this disclosure provides for a method for providing effective immunity to a patient from microbes comprising oligomeric N-acetyl-β-(1→6)-glucosamine groups in their cell wall which method comprises administering a compound of formula I or II above.

[0015] In one example, this disclosure provides for a method for providing effective immunity to a patient from microbes comprising oligomeric N-acetyl-β-(1→6)-glucosamine

groups in their cell wall which method comprises administering a pharmaceutical composition of this disclosure as described above to said patient.

[0016] In one example, the compounds of this disclosure include those where x is from 33 to 39. In another example, the compounds of this disclosure include those where x is from 35-38.

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



Example	Y	C	Percent N-acetylated	*x*	Percent monomer
A	2	Tetanus toxoid	0%	31	90%
B	3	Tetanus toxoid	0%	36	95%
C	6	Tetanus toxoid	12.5% (1 of 8)	33	95%
D	10	Tetanus toxoid	25% (3 of 12)	30	>95%
E	3	Tetanus toxoid	20% (1 of 5)	34	>95%
F	4	Tetanus toxoid	33% (2 of 6)	33	90%
G	3	Tetanus toxoid	20% (2 of 5)	30	>90%
H	3	Tetanus toxoid	0%	35	>99%

[0018] In one example, the compositions of this disclosure comprise no more about 3 weight percent of low molecular weight amino groups based on the total weight of the compound of formula I or II.

[0019] In one example, this disclosure provides methods for providing immunity to a patient against microbes comprising oligosaccharide β-(1→6)-glucosamine groups in their cell wall which methods comprise administering to said patient an effective amount of a compound represented by formula I:

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

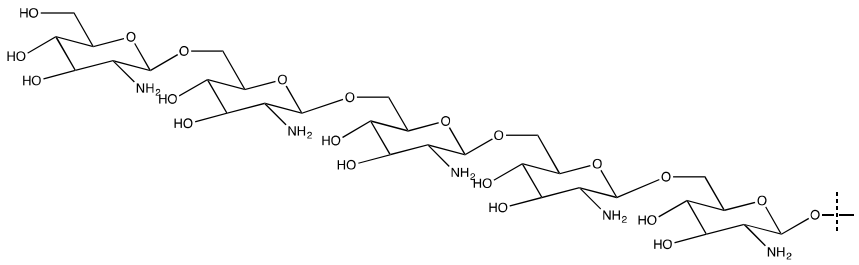
wherein A, B, C and x are as defined above and elsewhere herein.

[0020] In one example, this disclosure provides methods for providing immunity to a patient against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall comprising administering to said patient an effective amount of the compounds of formula I as defined above and elsewhere herein, in which y is 2, 3, or 4 as well as mixtures thereof.

[0021] In one example, this disclosure provides methods for providing immunity to a patient against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall which methods comprise administering to said patient an effective amount of the compounds of 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 and elsewhere herein.

[0022] In one example, this disclosure provides methods for providing effective immunity to a subject against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall which methods comprise administering to said patient an effective amount of the pharmaceutical composition of a pharmaceutically acceptable diluent and an effective amount of the compound of formula I:

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

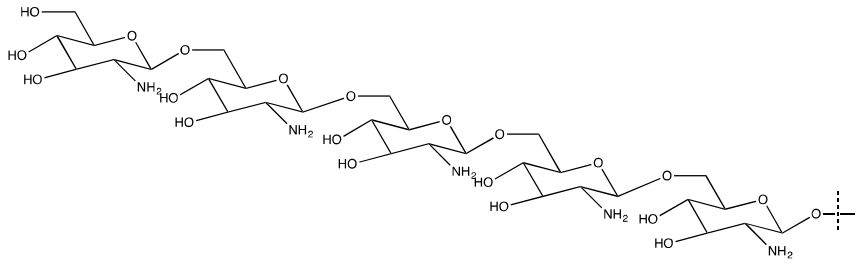
wherein A, B, C and x are as defined above and elsewhere herein.

[0023] In one example, this disclosure provides methods for providing effective immunity to a subject against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall, which methods comprise administering to said patient an effective amount of the pharmaceutical composition of a pharmaceutically acceptable diluent and an effective amount of the compound of formula I as defined above and elsewhere herein, in which y is 2, 3, or 4.

[0024] In one example, this disclosure provides methods for providing effective immunity to a subject against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall, which methods comprise administering to said patient an effective amount of the pharmaceutical composition of a pharmaceutically acceptable diluent and an effective amount of the compound of 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 and elsewhere herein.

[0025] In one example, this disclosure provides methods for providing effective immunity to a subject against microbes comprising N-acetyl oligosaccharide β-(1→6)-glucosamine groups in their cell wall which methods comprise administering to said patient an effective amount of the above compounds as pharmaceutical compositions with a pharmaceutically acceptable diluent and an effective amount of the compound, wherein said patient has a white blood count of at least 2,000.

[0026] In some examples, in one or more of the aforementioned methods, the pharmaceutical compositions can include, for example, no more than about 3 weight percent of low molecular weight amino compounds, or in alternative examples, less than 1 weight percent of low molecular weight amino compounds, and any subvalue or subrange from 3 weight percent to zero.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1 illustrates the ^{1}H NMR for compound 17 (as described below).

[0028] Figure 2 illustrates the ^{13}C NMR for compound 17.

[0029] Figure 3 illustrates the HPLC spectrum for the separation of tetanus toxoid monomer from oligomers and low molecular weight amino compounds.