

**CLAIMS:**

1. A method for producing a glycoconjugate immunogen, the method comprising:
  - (a) providing a carbohydrate antigen covalently linked to a terminal alkene (alkenyl carbohydrate antigen), the terminal alkene being directly conjugatable to a thiol group via a thiol-ene reaction;
  - (b) providing a carrier protein having one or more free thiol groups; and
  - (c) performing a photocatalytic thiol-ene reaction to directly conjugate the carbohydrate antigen to the carrier protein at the one or more free thiol groups, thereby producing the glycoconjugate immunogen;wherein the carrier protein is immunogenic when administered to a subject, and wherein conjugation of the carbohydrate antigen to the carrier protein increases the immunogenicity of the carbohydrate antigen upon administration to the subject, as compared to administration of the unconjugated carbohydrate antigen.
2. The method of claim 1, wherein the alkenyl carbohydrate antigen is water-soluble.
3. The method of claim 1 or 2, wherein said photocatalytic thiol-ene reaction is performed under reaction conditions that avoid carrier protein denaturation, and/or that retain the carrier protein's activity, antigenicity, and/or structure.
4. The method of any one of claims 1 to 3, wherein said photocatalytic thiol-ene reaction is performed in the absence of any organic solvent, or wherein said photocatalytic thiol-ene reaction is performed in the presence of an organic solvent at a concentration sufficiently low to avoid carrier protein denaturation.
5. The method of any one of claims 1 to 4, wherein said photocatalytic thiol-ene reaction is performed in the presence of a catalyst.
6. The method of claim 5, wherein the catalyst is:
  - (a) a water-soluble catalyst, such as a water-soluble free radical-generating azo compound; 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (Vazo 44 or VA-044); 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH); metals or metal ions having photoinitiator

activity; a peroxide; *tert*-butyl hydroperoxide; benzoylperoxide; ammonium persulfate; or any derivative thereof having photoinitiator activity; or

- 5 (b) a water-insoluble catalyst, such as a water-insoluble free radical-generating azo compound, 2,2-dimethoxy-2-phenylacetophenone (DMPA), azobisisobutyronitrile (AIBN), 2,2'-azobis(2-methylpropionitrile), 4,4'-Azobis(4-cyanopentanoic acid) (ACVA), 1,1'-azobis(cyanocyclohexane) (ACHN), diazenedicarboxylic acid bis(*N,N*-dimethylamide) (TMAD); azodicarboxylic acid dipiperidide (ADD), or any derivative thereof having photoinitiator activity.

10 7. The method of any one of claims 1 to 6, wherein said photocatalytic thiol-ene reaction comprises irradiation under short-wave ultraviolet light.

8. The method of claim 7, wherein said photocatalytic thiol-ene reaction comprises irradiation at about 254 nm.

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9. The method of any one of claims 1 to 6, wherein said photocatalytic thiol-ene reaction comprises irradiation under long-wave ultraviolet light.

10. The method of claim 9, wherein said photocatalytic thiol-ene reaction comprises irradiation at about 355 nm or 365 nm.

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11. The method of any one of claims 1 to 10, wherein said photocatalytic thiol-ene reaction comprises reacting between 1 to 200 molar equivalents of the alkenyl carbohydrate antigen per free thiol group of the carrier protein; and/or wherein said photocatalytic thiol-ene reaction is performed for 10 to 300, 10 to 270, 10 to 240, 10 to 210, 10 to 180, 10 to 150, 10 to 120, 10 to 90, 10 to 60, or 10 to 30 minutes, and/or for a sufficient time to achieve at least a 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50-fold reduction in total free thiol concentration in the carrier protein.

12. The method of any one of claims 1 to 11, wherein said photocatalytic thiol-ene reaction is performed at a pH between about 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, or 4.0, and about 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, or 10, and/or at a pH that avoids carrier protein denaturation.

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13. The method of any one of claims 1 to 12, wherein said carbohydrate antigen, following conjugation to the carrier protein, is not cleavable from the carrier protein by an endogenous enzyme of the subject.
- 5 14. The method of any one of claims 1 to 13, wherein said alkenyl carbohydrate antigen is covalently linked to the terminal alkene, and/or the carbohydrate antigen is conjugated to the carrier protein, via a glycosidic bond, such as is an O-glycosidic bond, an S-glycosidic bond, an N-glycosidic bond, or a C-glycosidic bond, or a bond obtained by reductive amination, such as between an allyl amine and a reducing sugar.
- 10 15. The method of any one of claims 1 to 14, wherein the carbohydrate antigen comprises a B cell epitope, and/or induces a humoral immune response in the subject; and/or comprises T cell epitope, and/or induces a cell-mediated immune response in the subject.
- 15 16. The method of any one of claims 1 to 15, wherein the carbohydrate antigen is or comprises a tumor associated carbohydrate antigen (TACA).
17. The method of claim 16, wherein the TACA is, is from, or comprises: Tn, S-Tn, *Thomsen-Friedenreich* (TF), (2,3)-S-TF, (2,6)-S-TF, Globo H, GD2, GD3, GM2, GM3, N-glycolyl-GM3, Lea, sLea, Lex, sLex, or any combination thereof.
- 20 18. The method of any one of claims 1 to 17, wherein said photocatalytic thiol-ene reaction conjugates at least two of the same carbohydrate antigen or more than one type of carbohydrate antigen to the carrier protein, thereby producing a multi-valent glycoconjugate immunogen.
- 25 19. The method of claim 18, wherein said multi-valent glycoconjugate immunogen comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more of the same or different types of carbohydrate antigens conjugated to the carrier protein.
- 30 20. The method of any one of claims 1 to 19, wherein the carbohydrate antigen is or comprises a viral polysaccharide antigen, or a bacterial capsular polysaccharide (CPS).

21. The method of claim 20, wherein the bacterial CPS is, is from, or comprises a Pneumococcal and/or Streptococcal polysaccharide serotype, meningococcal CPS, or influenza (such as influenza type a or b) CPS.
- 5 22. The method of any one of claims 1 to 21, wherein the carbohydrate antigen in (a) is linked to the terminal alkene via a linker.
23. The method of any one of claims 1 to 22, wherein the carrier protein comprises one or more cysteine residues having the one or more free thiol groups, or optionally is engineered to add one or more  
10 further cysteine residues, for example at the amino terminus, the carboxy terminus, or a solvent-accessible position of the carrier protein.
24. The method of any one of claims 1 to 23, wherein the carrier protein comprises a human T cell epitope, and/or induces a cell-mediated immune response in the subject.
- 15 25. The method of any one of claims 1 to 24, wherein the carrier protein is, is from, or comprises: Tetanus Toxoid (TT), Diphtheria Toxoid (DT), cross-reacting material 197 (CRM197), Meningococcal Outer Membrane Protein Complex (OMPC), H. Influenzae Protein D (HiD), a cytokine, an immunogenic peptide such as Tetanus Toxin 831-844 (**SEQ ID NO: 1 or 2**), albumin (such as bovine serum albumin or  
20 human serum albumin), or an immunogenic fragment thereof.
26. The method of any one of claims 1 to 25, wherein:
- (i) the carrier protein, prior to or together with performing the photocatalytic thiol-ene reaction, is pre-treated with a reducing agent, such as dithiothreitol (DTT), tris(2-  
25 carboxyethyl)phosphine (TCEP), 2-mercaptoethylamine-HCl, or 2-mercaptoethanol;
- (ii) the carrier protein, prior to or together with performing the photocatalytic thiol-ene reaction, is pre-treated with a thiolating agent, such as 2-iminothiolane or N-hydroxysuccinimide dithiopropionate (DPS);
- (iii) the carrier protein, prior to or together with performing the photocatalytic thiol-ene reaction, is pre-treated with a thiolating agent, such as 2-iminothiolane or N-hydroxysuccinimide  
30 dithiopropionate (DPS), and subsequently pre-treated with a reducing agent;
- (iv) the carrier protein is a protein having one or more disulfide bridges, and wherein the one or more disulfide bridges remain unaffected following said photocatalytic thiol-ene reaction;

- (v) the carrier protein is a protein having one or more disulfide bridges, and wherein the carrier protein is pre-treated with a reducing agent to expose one or more additional free thiol groups for conjugation to the carbohydrate antigen; or
- (iv) the carrier protein is a protein having one or more disulfide bridges, and wherein the carrier protein is pre-treated with a thiolating agent (such as 2-iminothiolane or N-hydroxysuccinimide dithiopropionate (DPS)) to expose one or more additional free thiol groups for conjugation to the carbohydrate antigen, and subsequently pre-treated with a reducing agent.
27. The method of any one of claims 1 to 26, wherein the total number of carbohydrate antigens comprised in the glycoconjugate immunogen is equal to the number of free thiol groups available on the carrier protein prior to conjugation.
28. The method of any one of claims 1 to 27, wherein the glycoconjugate immunogen induces a cell-mediated immune response to the carbohydrate antigen upon administration to the subject.
29. The method of any one of claims 1 to 28, further comprising: (d) purifying the glycoconjugate immunogen.
30. A method for producing a glycoconjugate vaccine or an adaptive immune response-triggering composition, the method comprising formulating the glycoconjugate immunogen prepared by the method of any one of claims 1 to 29 with a pharmaceutically acceptable excipient, and/or an adjuvant.
31. The method of claim 30, wherein the adjuvant is or comprises: an inorganic compound, a mineral oil, a microbial derivative, a plant derivative, a cytokine, squalene, alum, aluminum hydroxide, aluminum phosphate, calcium phosphate hydroxide, a toll-like receptor agonist, an immunostimulatory polynucleotide (such as CPG), an immunostimulatory lipid, Freund's adjuvant, RIBI's adjuvant, QS-21, muramyl dipeptide, TiterMax, Steviune, Stimune, or any combination thereof.
32. A synthetic glycoconjugate immunogen comprising one or more carbohydrate antigens and an immunogenic carrier protein having one or more solvent-accessible cysteine residues, wherein the one or more carbohydrate antigens are linked to the immunogenic carrier protein at the one or more solvent-accessible cysteine residues, and wherein conjugation of the one or more carbohydrate antigens to the

immunogenic carrier protein increases the immunogenicity of the one or more carbohydrate antigens upon administration to a subject, as compared to administration of the unconjugated carbohydrate antigen.

33. The synthetic glycoconjugate immunogen of claim 32, wherein the one or more carbohydrate  
5 antigens are linked to the one or more solvent-accessible cysteine residues via a linker.

34. The synthetic glycoconjugate immunogen of claim 32, wherein the one or more carbohydrate antigen is linked to the linker via a bond as defined in claim 13 or 14.

10 35. The synthetic glycoconjugate immunogen of any one of claims 32 to 34, wherein the one or more carbohydrate antigens is/are as defined in claim 2, 15, 16, 17, 20, or 21.

36. The synthetic glycoconjugate immunogen of any one of claims 32 to 35, which is the multi-valent glycoconjugate immunogen as defined in claim 18 or 19.

15 37. The synthetic glycoconjugate immunogen of any one of claims 32 to 36, wherein the carrier protein is as defined in claim 23, 24, 25, or 26.

38. The synthetic glycoconjugate immunogen of any one of claims 32 to 37, wherein the total number  
20 of carbohydrate antigens comprised in the glycoconjugate immunogen is equal to the number of solvent-accessible cysteine residues on the carrier protein.

39. The synthetic glycoconjugate immunogen of any one of claims 32 to 38, which induces a cell-mediated immune response to the carbohydrate antigen upon administration to the subject.

25 40. The synthetic glycoconjugate immunogen of any one of claims 32 to 39, which is prepared by the method of any one of claims 1 to 29.

41. A glycoconjugate vaccine produced by the method of claim 30 or 31, and/or comprising the  
30 synthetic glycoconjugate immunogen of any one of claims 32 to 40, and a pharmaceutically acceptable excipient, and/or an adjuvant.

42. The glycoconjugate vaccine of claim 41, which is a prophylactic vaccine or a therapeutic vaccine.

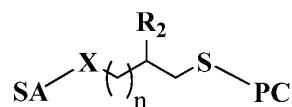
43. A method of immunizing, vaccinating, or treating a subject comprising administering to the subject the glycoconjugate immunogen produced by the method of any one of claims 1 to 29, the glycoconjugate vaccine produced by the method of claim 30 or 31, the synthetic glycoconjugate immunogen of any one of claims 32 to 40, or the glycoconjugate vaccine of claim 41 or 42.

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44. The synthetic glycoconjugate immunogen of any one of claims 32 to 40, or the glycoconjugate vaccine of claim 41 or 42, for use in immunizing, vaccinating, or treating a subject having a disease, or for detecting the presence of an antibody that specifically binds to the glycoconjugate or for detecting said immunization, vaccination, or treatment (e.g., in a biological sample from the subject).

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45. A synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein each linker has the structure:



wherein:

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- SA is a sugar antigen or a portion thereof;

- S-PC is a carrier protein;

- X is O, S, NR<sub>1</sub>, or CH<sub>2</sub>;

- R<sub>1</sub> is -H, -COH, -COCH<sub>3</sub>, or -COEt;

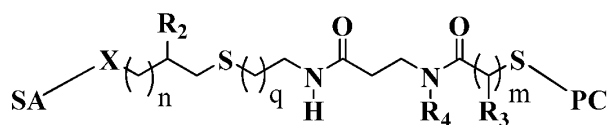
- n is 1, 2, 3, 4, or 5; and

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- R<sub>2</sub> is H or Me;

or a stereoisomer thereof.

46. A synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein each linker has the structure:



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wherein:

- SA is a sugar antigen or a portion thereof;

- S-PC is a carrier protein;

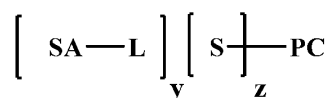
- X is S, NR<sub>1</sub>, CH<sub>2</sub> or O;

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- R<sub>1</sub> is -H, -COH, -COMe, or -COEt;

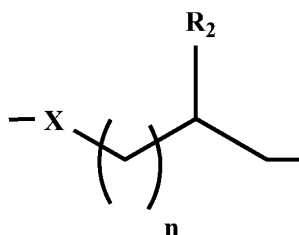
- **n** is 1, 2, 3, 4, or 5;
  - **R<sub>2</sub>** is H or Me;
  - **q** is 1, 2, 3, 4, or 5;
  - **R<sub>3</sub>** and **R<sub>4</sub>** are each a hydrogen atom and **m** is 1, 2, 3, 4 or 5, or **R<sub>3</sub>** and **R<sub>4</sub>** form together a radical -CO-CH<sub>2</sub>- or a radical -CO-CH<sub>2</sub>-CH<sub>2</sub>- with the carbonyl linked to the nitrogen atom, and **m** is 1;
- or a stereoisomer thereof.

47. A synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein the synthetic glycoconjugate immunogen has the structure:



wherein

- **y** is at least 1;
- **SA** is selected from the group consisting of one or more sugar antigens or immunogenic portions thereof, and when **y** is more than 1, **SA** are identical or different;
- [**S**]<sub>**z**</sub>-**PC** is a carrier protein having one or more sulfur atoms originating from the one or more free thiol groups;
- **z** is at least 1 and is at least equal to **y**; and
- **L** is a linker selected from the group consisting of linkers having the structure:



wherein:

- **X** is O, S, NR<sub>1</sub>, or CH<sub>2</sub>;
  - **R<sub>1</sub>** is -H, -COH, -COCH<sub>3</sub>, or -COEt;
  - **n** is 1, 2, 3, 4, or 5; and
  - **R<sub>2</sub>** is H or Me; and
- when **y** is more than 1, **L** are identical or different;

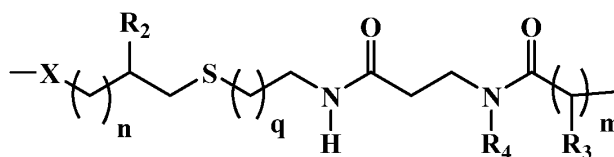
or a stereoisomer thereof.

48. A synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein the synthetic glycoconjugate immunogen has the structure:



wherein

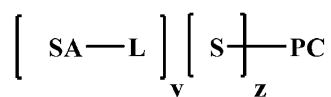
- $y$  is at least 1;
- **SA** is selected from the group consisting of sugar antigens or immunogenic portions thereof, and when  $y$  is more than 1, **SA** are identical or different;
- $[\text{S}]_z\text{-PC}$  is a carrier protein having one or more sulfur atoms originating from  $z$  free thiol groups;
- $z$  is at least equal to  $y$ ; and
- **L** is a linker selected from the group consisting of linkers having the structure:



wherein:

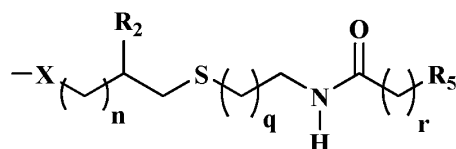
- **X** is S,  $\text{NR}_1$ ,  $\text{CH}_2$  or O;
  - $\text{R}_1$  is -H, -COH, -COMe, or -COEt;
  - $n$  is 1, 2, 3, 4, or 5;
  - $\text{R}_2$  is H or Me;
  - $q$  is 1, 2, 3, 4, or 5;
  - $\text{R}_3$  and  $\text{R}_4$  are each a hydrogen atom and  $m$  is 1, 2, 3, 4 or 5, or  $\text{R}_3$  and  $\text{R}_4$  form together a radical -CO-CH<sub>2</sub>- or a radical -CO-CH<sub>2</sub>-CH<sub>2</sub>- with the carbonyl linked to the nitrogen atom, and  $m$  is 1; and
  - when  $y$  is more than 1, **L** are identical or different;
- or a stereoisomer thereof.

49. A synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein the synthetic glycoconjugate immunogen has the structure:



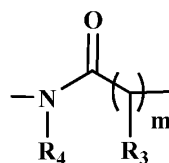
5 wherein

- $y$  is at least 1;
- $\text{SA}$  is selected from the group consisting of sugar antigens or immunogenic portions thereof, and when  $y$  is more than 1,  $\text{SA}$  are identical or different;
- $[\text{S}]_z\text{-PC}$  is a carrier protein having one or more sulfur atoms originating from  $z$  free thiol groups;
- $z$  is at least equal to  $y$ ; and
- $\text{L}$  is a linker selected from the group consisting of linkers having the structure:



wherein

- $\text{X}$  is S,  $\text{NR}_1$ ,  $\text{CH}_2$  or O;
- $\text{R}_1$  is -H, -COH, -COMe, or -COEt;
- $n$  is 1, 2, 3, 4, or 5;
- $\text{R}_2$  is H or Me;
- $q$  is 1, 2, 3, 4, or 5;
- $r$  is 1, 2, 3, 4 or 5;
- $\text{R}_5$  is S-PC, a covalent bond, or a radical of structure:

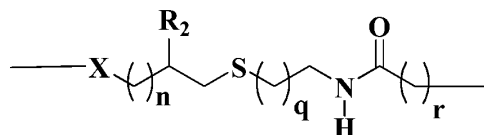


wherein  $\text{R}_3$  and  $\text{R}_4$  are each a hydrogen atom and  $m$  is 1, 2, 3, 4 or 5, or  $\text{R}_3$  and  $\text{R}_4$  form together a radical -CO-CH<sub>2</sub>- or a radical -CO-CH<sub>2</sub>-CH<sub>2</sub>- with the carbonyl linked to the nitrogen atom, and  $m$  is 1; and

when  $y$  is more than 1,  $\text{L}$  are identical or different;

or a stereoisomer thereof.

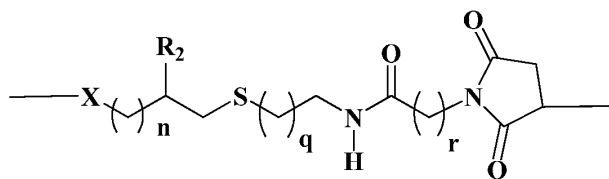
50. The synthetic glycoconjugate immunogen of claim 49, wherein the linker has the structure:



wherein

- 5                   - **X** is S, NR<sub>1</sub>, CH<sub>2</sub> or O;  
                   - **R**<sub>1</sub> is -H, -COH, -COMe, or -COEt;  
                   - **n** is 1, 2, 3, 4, or 5;  
                   - **R**<sub>2</sub> is H or Me;  
                   - **q** is 1, 2, 3, 4, or 5; and  
 10               - **r** is 1, 2, 3, 4 or 5.

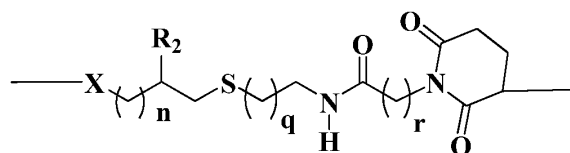
51. The synthetic glycoconjugate immunogen of claim 49, wherein the linker has the structure:



wherein

- 15               - **X** is S, NR<sub>1</sub>, CH<sub>2</sub> or O;  
                   - **R**<sub>1</sub> is -H, -COH, -COMe, or -COEt;  
                   - **n** is 1, 2, 3, 4, or 5;  
                   - **R**<sub>2</sub> is H or Me;  
                   - **q** is 1, 2, 3, 4, or 5; and  
 20               - **r** is 1 or 2.

52. The synthetic glycoconjugate immunogen of claim 49, wherein the linker has the structure:



wherein

- 25               - **X** is S, NR<sub>1</sub>, CH<sub>2</sub> or O;  
                   - **R**<sub>1</sub> is -H, -COH, -COMe, or -COEt;

- **n** is 1, 2, 3, 4, or 5;
- **R<sub>2</sub>** is H or Me;
- **q** is 1, 2, 3, 4, or 5; and
- **r** is 1 or 2.

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53. The synthetic glycoconjugate immunogen of any one of claims 47 to 52, wherein **y** is an integer varying from 1 to 50, 1 to 40, 1 to 30, 1 to 20, or 1 to 10.

54. The synthetic glycoconjugate immunogen of any one of claims 45 to 53, wherein said sugar antigen is the carbohydrate antigen as defined in claim 15, 16, 17, 20, or 21.

55. The synthetic glycoconjugate immunogen of any one of claims 45 to 54, wherein said carrier protein is the carrier protein as defined in claim 23, 24, or 25.

56. The synthetic glycoconjugate immunogen of any one of claims 45 to 55, which is a multi-valent glycoconjugate immunogen.

57. The synthetic glycoconjugate immunogen of claim 56, wherein said multi-valent glycoconjugate immunogen comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more of the same or different types of carbohydrate antigens conjugated to the carrier protein.

58. The synthetic glycoconjugate immunogen of any one of claims 45 to 57, wherein the glycoconjugate immunogen induces a cell-mediated immune response to the carbohydrate antigen upon administration to a subject.

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59. A vaccine comprising the synthetic glycoconjugate immunogen of any one of claims 45 to 58, and a pharmaceutically acceptable excipient and/or an adjuvant.

60. The vaccine of claim 59, wherein the adjuvant is as defined in claim 31.

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61. Use of the glycoconjugate immunogen prepared by the method of any one of claims 1 to 29, or the synthetic glycoconjugate immunogen as defined in any one of claim 32 to 40 and 45 to 58, for the manufacture of a vaccine.

62. Use of the glycoconjugate immunogen prepared by the method of any one of claims 1 to 29, the glycoconjugate vaccine produced by the method of claim 30 or 31, the synthetic glycoconjugate immunogen as defined in any one of claim 32 to 40 and 45 to 58, or the vaccine of claim 59 or 60, for the treatment of a subject having a disease associated with increased expression of said one or more carbohydrate or sugar antigens.

63. Use of the glycoconjugate immunogen prepared by the method of any one of claims 1 to 29, the glycoconjugate vaccine produced by the method of claim 30 or 31, the synthetic glycoconjugate immunogen as defined in any one of claim 32 to 40 and 45 to 58, or the vaccine of claim 59 or 60, for producing an antibody that specifically binds to the glycoconjugate immunogen.

64. Use of the glycoconjugate immunogen prepared by the method of any one of claims 1 to 29, the glycoconjugate vaccine produced by the method of claim 30 or 31, the synthetic glycoconjugate immunogen as defined in any one of claim 32 to 40 and 45 to 58, for detecting an antibody that specifically binds to the glycoconjugate immunogen.

65. A method for producing a glycoconjugate, the method comprising:

- (a) providing a carbohydrate antigen covalently linked to a terminal alkene (alkenyl carbohydrate antigen), the terminal alkene being directly conjugatable to a thiol group via a thiol-ene reaction;
- (b) providing a conjugate material suitable for conjugation to the carbohydrate antigen via a thiol-ene reaction as described herein, said conjugate material having one or more free thiol groups; and
- (c) performing a photocatalytic thiol-ene reaction to directly conjugate the carbohydrate antigen to the conjugate material at the one or more free thiol groups, thereby producing the glycoconjugate.

66. The method of claim 65, wherein the conjugate material is or comprises a polymer, a polypeptide, a carrier protein as defined in any one of preceding claims, a solid support, a particle, or any other material having a free thiol group suitable for conjugation to the carbohydrate antigen via a thiol-ene reaction as described herein.

67. The method of claim 65 or 66, further comprising one or more features as defined in any one of claims 1 to 64.

68. Use of a glycoconjugate produced by the method of any one of claims 65 to 67, for detecting or screening for the presence of an antibody that specifically binds to a carbohydrate antigen or a tumor-circulating cell comprising a carbohydrate antigen, or for detecting the presence of antibodies resulting from an immunization or vaccination with a carbohydrate antigen.

69. The use of claim 68, wherein the detection or screening is performed via any suitable detection method such as an immunosorbent assay, ELISA, microarray, or immunoblot analysis.

70. A method of treating a subject comprising administering a glycoconjugate or glycoconjugate immunogen as defined in any preceding claims or produced by a method as defined by any preceding claims, to generate an immune response in said subject to a carbohydrate antigen, and screening a biological sample from said subject for the presence of antibodies that specifically binds to the carbohydrate antigen.

71. The method of claims 70, further comprising one or more features as described in preceding claims.