CLAIMS:

[Claim 1]. A method for producing a glycoconjugate immunogen, the method comprising:

(a) providing a water-soluble 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 and wherein the alkenyl carbohydrate antigen is an unprotected, water-soluble alkenyl carbohydrate antigen;

(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 photocatalytic thiol-ene reaction is performed:

(i) at a pH between 6 and 8;

(ii) under reaction conditions that avoid carrier protein denaturation, and/or that retain the carrier protein's activity, antigenicity, and/or structure; and

(iii) in the absence of any organic solvent, or in the presence of an organic solvent at a concentration sufficiently low to avoid carrier protein denaturation;

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, and wherein the carbohydrate antigen comprises a B cell epitope capable of inducing a humoral immune response when administered to a subject.

[Claim 2]. The method of claim 1, wherein said photocatalytic thiol-ene reaction is performed at a pH between 6.5 and 7.5~~under reaction conditions that avoid carrier protein denaturation, and/or that retain the carrier protein's activity, antigenicity, and/or structure~~.

~~3. The method of claim 1 or 2, 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.~~

[Claim ~~4~~3]. The method of ~~any one of~~ claim~~s~~ 1 ~~t~~or ~~3~~2, wherein said photocatalytic thiol-ene reaction is performed in the presence of a water-soluble catalyst.

[Claim ~~5~~4]. The method of claim ~~4~~3, wherein the water-soluble catalyst is:

a 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.

[Claim ~~6~~5]. The method of ~~any one of~~ claim~~s~~ 1 ~~t~~or ~~3~~2, wherein said photocatalytic thiol-ene reaction is performed in the presence of a water-insoluble catalyst.

[Claim ~~7~~6]. The method of claim ~~6~~5, wherein the water-insoluble catalyst is: 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.

[Claim ~~8~~7]. The method of any one of claims 1 to ~~7~~6, wherein said photocatalytic thiol-ene reaction comprises irradiation under ultraviolet light.

[Claim ~~9~~8]. The method of any one of claims 1 to ~~8~~7, 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.

[Claim ~~10~~9]. The method of any one of claims 1 to ~~9~~8, wherein said photocatalytic thiol-ene reaction is performed at a pH that avoids carrier protein denaturation.

[Claim 1~~1~~0]. The method of any one of claims 1 to ~~10~~9, wherein said carbohydrate antigen, following conjugation to the carrier protein, is not cleavable from the carrier protein by an endogenous enzyme of the subject.

[Claim 1~~2~~1]. The method of any one of claims 1 to 1~~0~~1, 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 as between an allyl amine and a reducing sugar.

[Claim 123]. The method of any one of claims 1 to 112, wherein the carbohydrate antigen is or comprises a tumor associated carbohydrate antigen (TACA), a viral polysaccharide antigen, or a bacterial capsular polysaccharide (CPS).

[Claim 134]. The method of claim 123, 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, LeAa, sSLeAa, LeXx, SsLeXx, or any combination thereof; and wherein the bacterial capsular polysaccharide (CPS) is, is from, or comprises a Pneumococcal and/or Streptococcal polysaccharide serotype, meningococcal CPS, or influenza CPS.

[Claim 145]. The method of any one of claims 1 to 134, wherein said photocatalytic thiol-ene reaction conjugates more than one type of carbohydrate antigen to the carrier protein.

[Claim 156]. The method of any one of claims 1 to 145, wherein the carbohydrate antigen in (a) is linked to the terminal alkene via a linker.

[Claim 167]. The method of any one of claims 1 to 156, wherein the carrier protein provided in step (b) comprises

(i) a carrier protein comprising one or more cysteine residues having the one or more free thiol groups;

(ii) a carrier protein engineered to add one or more further cysteine residues at a solvent-accessible position of the carrier protein;

(iii) a carrier protein treated with a thiolating agent;

(iv) a carrier protein treated with a reducing agent; or

(v) any combination of (i) to (iv).

[Claim 178]. The method of any one of claims 1 to 167, 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, or an immunogenic fragment thereof.

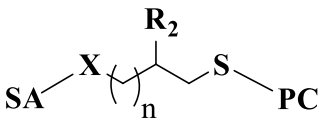
[Claim 189]. A method for producing a glycoconjugate vaccine or an adaptive immune response-triggering composition, the method comprising formulating the glycoconjugate immunogen prepared by the method of any one of claims 1 to 18 with a pharmaceutically acceptable excipient, and/or an adjuvant.

[Claim 1920]. A synthetic glycoconjugate immunogen prepared by the method of any one of claims 1 to 189, 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.

[Claim 201]. A synthetic glycoconjugate immunogen of claim 19, wherein the synthetic glycoconjugate immunogen is:

(a) 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:

(a) each linker has the structure:

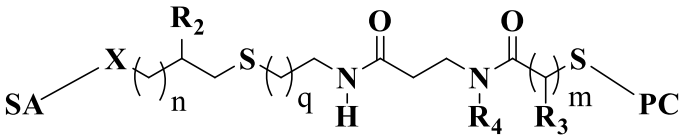


wherein:

- **SA** is a sugar antigen or a portion thereof;
- **S-PC** is a carrier protein;
- **X** is O, S, NR_1, or CH_2;
- **R_1** is -H, -COH, $-COCH_3$, or -COEt;
- **n** is 1, 2, 3, 4, or 5; and
- **R_2** is H or Me;

or a stereoisomer thereof; or

(b) 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:

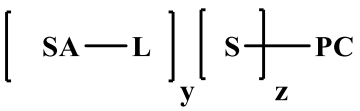


wherein:

- **SA** is a sugar antigen or a portion thereof;
- **S-PC** is a carrier protein;
- **X** is S, NR_1, CH_2 or O;
- **R_1** is -H, -COH, -COMe, or -COEt;
- **n** is 1, 2, 3, 4, or 5;
- **R_2** is H or Me;
- **q** is 1, 2, 3, 4, or 5;
- **R_3** and **R_4** are each a hydrogen atom and **m** is 1, 2, 3, 4 or 5, or **R_3** and **R_4** form together a radical $-CO-CH_2-$ or a radical $-CO-CH_2-CH_2-$ with the carbonyl linked to the nitrogen atom, and **m** is 1;

or a stereoisomer thereof; or

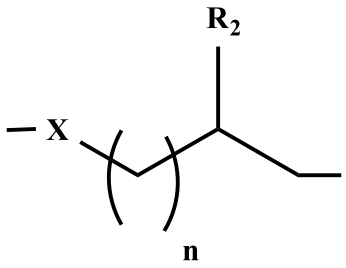
(c) a synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein the synthetic glycoconjugate immunogen has the structure:



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;
- **$[S]_z$-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:



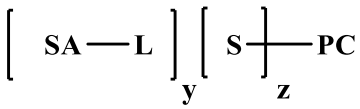
wherein:

- **X** is O, S, NR_1, or CH_2;
- **R_1** is -H, -COH, $-COCH_3$, or -COEt;
- **n** is 1, 2, 3, 4, or 5; and
- **R_2** is H or Me; and

when y is more than 1, **L** are identical or different;

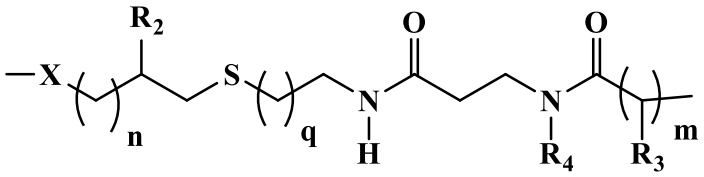
or a stereoisomer thereof; or

(d) 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;
- **$[S]_z$-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:



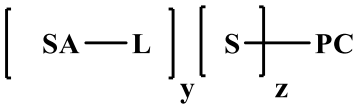
wherein:

- **X** is S, NR_1, CH_2 or O;
- **R_1** is -H, -COH, -COMe, or -COEt;
- **n** is 1, 2, 3, 4, or 5;
- **R_2** is H or Me;
- **q** is 1, 2, 3, 4, or 5;
- **R_3** and **R_4** are each a hydrogen atom and **m** is 1, 2, 3, 4 or 5, or **R_3** and **R_4** form together a radical -CO-CH_2- or a radical -CO-CH_2-CH_2- with the carbonyl linked to the nitrogen atom, and **m** is 1; and

when y is more than 1, **L** are identical or different;

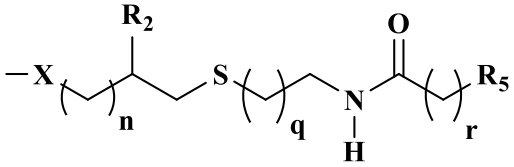
or a stereoisomer thereof; or

(e) 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:



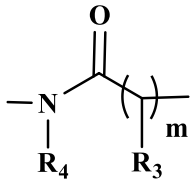
wherein

- **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;
- **$[S]_z$-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, NR_1, CH_2 or O;
- **R_1** is -H, -COH, -COMe, or -COEt;
- **n** is 1, 2, 3, 4, or 5;
- **R_2** is H or Me;
- **q** is 1, 2, 3, 4, or 5;
- **r** is 1, 2, 3, 4 or 5;
- **R_5** is S-PC, a covalent bond, or a radical of structure:

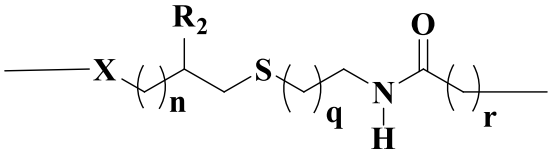


wherein **R_3** and **R_4** are each a hydrogen atom and **m** is 1, 2, 3, 4 or 5, or **R_3** and **R_4** form together a radical -CO-CH_2- or a radical -CO-CH_2-CH_2- with the carbonyl linked to the nitrogen atom, and **m** is 1; and

when y is more than 1, **L** are identical or different;

or a stereoisomer thereof; or

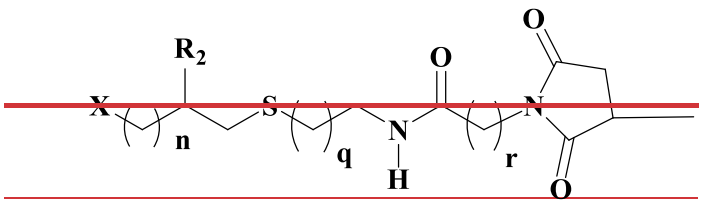
(~~g~~f) a synthetic glycoconjugate immunogen comprising one or more sugar antigens covalently linked to one or more free thiol groups of a carrier protein via a linker, wherein the linker has the structure:



wherein

- **X** is S, NR_1, CH_2 or O;
- **R_1** is -H, -COH, -COMe, or -COEt;
- **n** is 1, 2, 3, 4, or 5;
- **R_2** is H or Me;
- **q** is 1, 2, 3, 4, or 5; and
- **r** is 1, 2, 3, 4 or 5; or

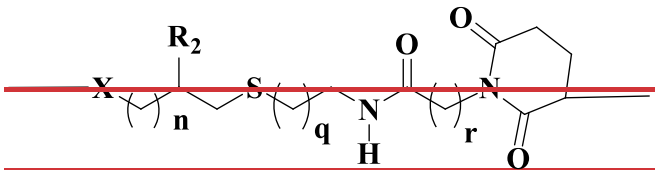
~~(h) the linker has the structure:~~



wherein

- X is S, NR_1, CH_2 or O;
- R_1 is H, COH, COMe, or COEt;
- n is 1, 2, 3, 4, or 5;
- R_2 is H or Me;
- q is 1, 2, 3, 4, or 5; and
- r is 1 or 2; or

(i) the linker has the structure:



wherein

- X is S, NR_1, CH_2 or O;
- R_1 is H, COH, COMe, or COEt;
- n is 1, 2, 3, 4, or 5;
- R_2 is H or Me;
- q is 1, 2, 3, 4, or 5; and
- r is 1 or 2.

[Claim 212]. The synthetic glycoconjugate immunogen of claim 201, wherein **y** is an integer varying from 1 to 50, 1 to 40, 1 to 30, 1 to 20, or 1 to 10.

[Claim 223]. The synthetic glycoconjugate immunogen of claim 201, wherein said sugar antigen is the carbohydrate antigen as defined in claim 123.

[Claim 234]. The synthetic glycoconjugate immunogen of claim 201, wherein said carrier protein is the carrier protein as defined in claim 178.

~~24. A vaccine comprising the synthetic glycoconjugate immunogen prepared by the method of any one of claims 1 to 19, and a pharmaceutically acceptable excipient and/or an adjuvant.~~

~~25. A method for producing a glycoconjugate, the method comprising:~~

~~(a) providing a water-soluble 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 and wherein the alkenyl carbohydrate antigen is an unprotected, water-soluble alkenyl carbohydrate antigen;~~

~~(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.~~

~~26. The method of claim 25, wherein the conjugate material is or comprises a polymer, a polypeptide, a carrier protein, 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.~~

~~27. An *in vitro* method 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, said method comprising contacting a biological sample isolated from a subject with the glycoconjugate produced by the method of claim 25; and the detecting or screening for the presence of antibodies bound to said glycoconjugate.~~

~~28. The method of claim 27, wherein the detection or screening is performed via any suitable detection method such as an immunosorbent assay, ELISA, microarray, or immunoblot analysis.~~