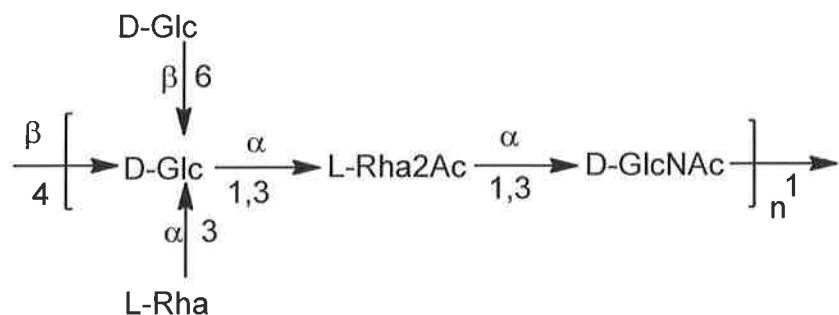


**What is Claimed is:**

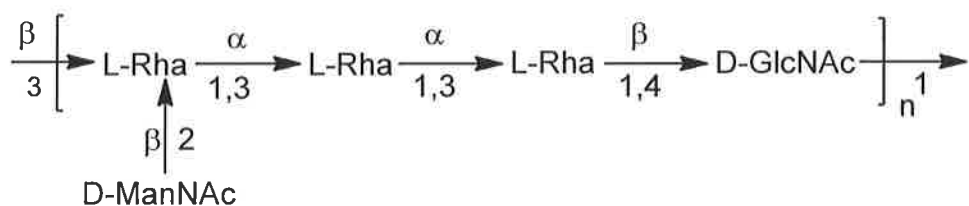
1. A composition comprising a bioconjugate of an *E.coli* O25B antigen covalently coupled to a carrier protein, wherein the *E.coli* O25B antigen comprises the structure of Formula O25B':



wherein n is an integer between 1 and 30.

2. The composition of claim 1, wherein the *E.coli* O25B antigen is covalently bound to an Asn residue in the carrier protein.
3. The composition of any one of claims 1-2, further comprising (i) an O1A bioconjugate, comprising an *E. coli* O1A antigen covalently bound to an Asn residue of a carrier protein; (ii) an O2 bioconjugate, comprising an *E. coli* O2 antigen covalently bound to an Asn residue of a carrier protein, and (iii) an O6 bioconjugate, comprising an *E. coli* O6 antigen covalently bound to an Asn residue of a carrier protein.
4. The composition of claim 3, wherein the O1A antigen, O6 antigen, and O2 antigen comprise the following formulas, respectively:

a. Formula O1A'



8. Use of a composition according to any one of claims 1-6 in the preparation of a medicament for inducing an immune response in a subject, preferably a human, against infection with extra-intestinal pathogenic *Escherichia coli*.
9. Use of a composition according to any one of claims 1-6 in the preparation of a medicament for inducing the production of opsonophagocytic antibodies in a subject, preferably a human, that are specific to extra-intestinal pathogenic *Escherichia coli*.
10. Use according to any one of claims 7-9, wherein the subject is at risk of developing a urinary tract infection, bacteremia, or sepsis.
11. A recombinantly engineered prokaryotic host cell, comprising:
  - a. a nucleotide sequence encoding:
    - i. dTDP-Glucose 4,6-dehydratase;
    - ii. dTDP-6-Deoxy-D-glucose 3,5-epimerase;
    - iii. Glucose-1-phosphate thymidyltransferase;
    - iv. dTDP-4-dehydrorhamnose 3,5-epimerase;
    - v. O antigen flippase;
    - vi. dTDP-Rha:Glc-Rha(Ac)-GlcNAc-UPP  $\alpha$ -1,3- rhamnosyltransferase;
    - vii. UDP-Glc:Glc-Rha(Ac)-GlcNAc-UPP  $\beta$ -1,6- glucosyltransferase;
    - viii. O antigen polymerase;
    - ix. O-acetyl transferase;
    - x. UDP-Glc:Rha-GlcNAc-UPP  $\alpha$ -1,3- glucosyltransferase and
    - xi. dTDP-Rha: GlcNAc-UPP  $\alpha$ -1,3- rhamnosyltransferase;
  - b. a nucleotide sequence encoding an oligosaccharyl transferase; and
  - c. a nucleotide sequence encoding a carrier protein comprising a consensus sequence Asp(Glu)-X-Asn-Z-Ser(Thr), wherein X and Z are independently selected from any natural amino acid except Pro (SEQ ID NO: 15), preferably the host cell is an *E. coli* host cell.

12. The recombinantly engineered prokaryotic host cell of claim 11 wherein at least one of the *waaL* gene, *gtrA* gene, *gtrB* gene, *gtrS* gene, or *rfb* cluster is deleted from or functionally inactivated in the genome of the host cell.

13. The recombinantly engineered prokaryotic host cell of claim 11 or 12, wherein the carrier protein is selected from the group consisting of detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM 197, maltose binding protein (MBP), Diphtheria toxoid, Tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A, clumping factor B, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* Sat protein, the passenger domain of *E. coli* Sat protein, *Streptococcus pneumoniae* Pneumolysin and detoxified variants thereof, *C. jejuni* AcrA, and *C. jejuni* natural glycoproteins, preferably the carrier protein is detoxified EPA.

14. A method of making a bioconjugate of an *E. coli* O25B antigen covalently coupled to a carrier protein, said method comprising:

- a. culturing the recombinantly engineered prokaryotic host cell of any one of claims 11-13; and
- b. purifying the bioconjugate.