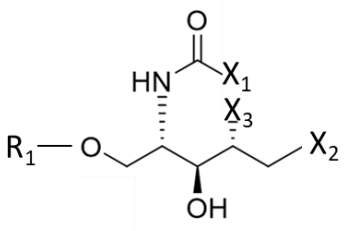
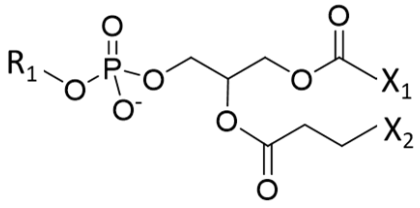
Claims

1. A pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components;

wherein the plurality of lipid components comprises a bi-functional compound comprising:




Formula 1; or




Formula 2;

wherein $R_1$ comprises a formula of $R_2$-$R_A$-, wherein $R_A$ is an attachment group and $R_2$ is a substituted or non-substituted glycosyl group, and wherein the attachment group comprises an aryl and an alkylamide, having a structure of –aryl-alkyl-NH-C(O)–, and the aryl moiety attaches to $R_2$;

wherein $X_1$ and $X_2$ are each independently hydrogen, $C_{1-30}$ alkyl, $C_{1-30}$ alkenyl, $C_{1-30}$ alkynyl, aryl, aryloxy, or a substituted version thereof, or

$–(CH_2)nX_4$, n is 0 to 30, and $X_4$ is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when $X_4$ is a heterocyclic group, the heterocyclic group comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N, or a combination thereof; and

wherein $X_3$ is hydrogen, $C_{1-6}$ alkyl, or hydroxyl;

wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) wherein the spike protein has a reduced and/or deleted glycan profile.

2. The pharmaceutical formulation of claim 1, wherein the spike protein comprises (1) an amino acid substitution of asparagine (N) to glutamine (Q) or (2) an amino acid deletion or addition at a N-linked glycosylation sequon (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue.

3. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 2, 16, 18, or 20, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence set forth in SEQ ID NO: 2, 16, 18 or 20; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, or 19 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17 or 19 respectively.

4. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, 22, 24, or 26, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 4, 22, 24, or 26, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation site; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 3, 21, 23, or 25 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 3, 21, 23, or 25 respectively.

5. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 6, 28, 30, or 32, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 6, 28, 30, or 32, and wherein the spike protein comprises a S2 subunit lacking at least one glycosylation site; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 5, 27, 29, or 31 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 5, 27, 29, or 31 respectively.

6. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 8 or 34, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 8 or 34, and wherein the spike protein comprises an S2 subunit comprising a single glycosylation site at N1194; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 7 or 33 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 7 or 33 respectively.

7. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 10 or 36, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 10 or 36, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation site and an amino acid substitution of N801Q; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 9 or 35 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 9 or 35 respectively.

8. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 12 or 38, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 12 or 38, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation site and an amino acid substitution of N1194Q; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 11 or 37 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 11 or 37 respectively.

9. The pharmaceutical formulation of claim 1 or claim 2, wherein

the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 14 or 40, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14 or 40, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation site and an amino acid substitution of N122Q, N165Q, N234Q, or a combination thereof; or

the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 13 or 39 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 13 or 39 respectively.

10. The pharmaceutical formulation of any one of claims 1 to 9, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group

consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46), GPKKSTNLVKNKC (SEQ ID NO: 47), NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.

11. The pharmaceutical formulation of any one of claims 1 to 10, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 53, 54, 55, 56, or 57, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 53, 54, 55, 56, or 57.

12. The pharmaceutical formulation of claim 11, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group consisting of:

SEQ ID NO: 58 - SSANNCTFEYVSQ;
SEQ ID NO: 59 - TESIVRFPNITNL;
SEQ ID NO: 60 - KPFERDISTEIYQAG;
SEQ ID NO: 61 - GPKKSTNLVKNKC;
SEQ ID NO: 62 - TEVPVAIHADQ;
SEQ ID NO: 63 - RVYSTGSNVFQTR;
SEQ ID NO: 64 - RRARSVASQS;
SEQ ID NO: 65 - DPSKPSKRSF;
SEQ ID NO: 66 - FIKQYGDCLGDI;
SEQ ID NO: 67 - ENQKLIANQFNS;
SEQ ID NO: 68 - GKIQDSLSSTA;
SEQ ID NO: 69 - NCDVVIGIVNNTVY;
SEQ ID NO: 70 - PELDSFKEELDKYFKNHTS;
SEQ ID NO: 71 - TSPDVDLGDISGINA;

SEQ ID NO: 72 - VNIQKEIDRLNEVA;
SEQ ID NO: 73 - NLNESLIDLQ; and
SEQ ID NO: 74 - LGKYEQYIKWP;

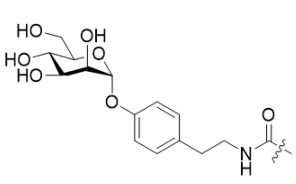
or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 58 to 74.

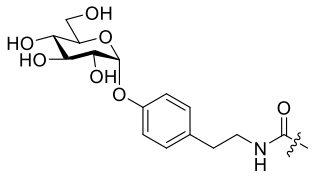
13. The pharmaceutical formulation of any one of claims 1 to 12, wherein the aryl moiety comprises 1 to 3 substituents, wherein the substituent is $C_{1-6}$ alkyl, halide, or $C_{1-6}$ alkyl halide.

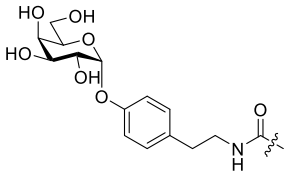
14. The pharmaceutical formulation of claim 1, wherein the glycosyl group comprises (1) mannoside, fucoside, or a combination thereof; or (2) a mono-mannoside, a di-mannoside, or a tri-mannoside.

15. The pharmaceutical formulation of any one of claims 1 to 14, wherein $R_1$ comprises a substituted glycosyl group, wherein the glycosyl group comprises 1 to 6 substituents, wherein the substituent is $C_{1-6}$ alkyl, $C_{1-6}$ alkenyl, halogen, $C_{1-6}$ alkyl halide, $C_{1-6}$ alkoxy, amine, nitro, $C_{1-6}$ alkyl amine, amide, azido, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

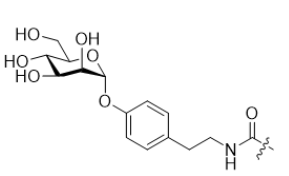
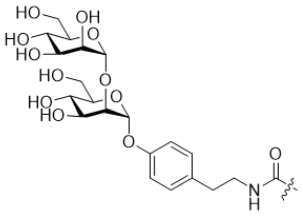
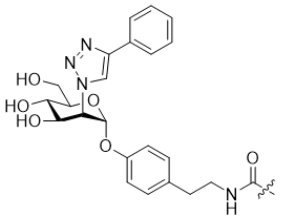
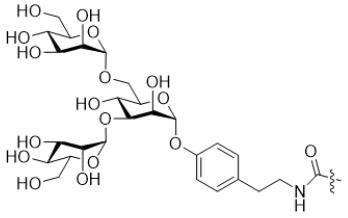
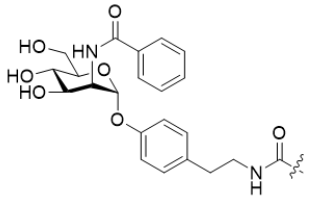
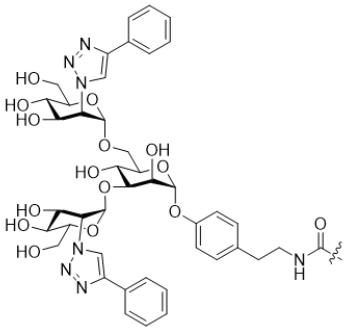
16. The pharmaceutical formulation of any one of claims 1 to 15, wherein $R_1$ is selected from the group consisting of:



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17. The pharmaceutical formulation of any one of claims 1 to 16, wherein at least one of $X_1$ and $X_2$ comprises a saturated hydrocarbon chain, and the saturated hydrocarbon chain comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons.

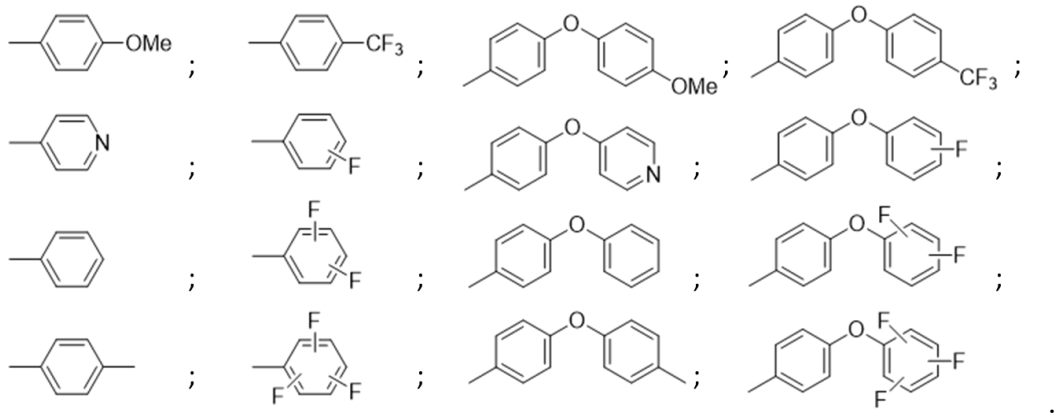
18. The pharmaceutical formulation of any one of claims 1 to 17, wherein $X_1$ and $X_2$ are each independently hydrogen, $C_{4\text{-}30}$ alkyl, $C_{4\text{-}30}$ alkenyl, $C_{4\text{-}30}$ alkynyl, aryl, aryloxy, or a substituted version thereof, or $–(CH_2)nX_4$, n is 4 to 30, and $X_4$ is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when $X_4$ is a

heterocyclic group, the heterocyclic group comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N, or a combination thereof.

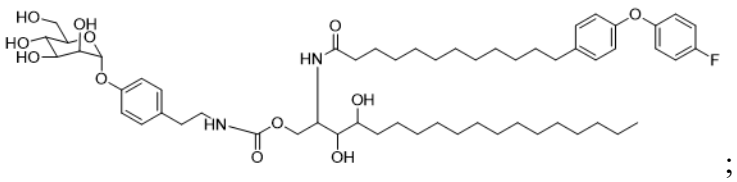
19. The pharmaceutical formulation of any one of claims 1 to 18, wherein $X_4$ is an aryl, aryloxy, heterocyclic group, cycloalkyl, heterocycloalkyl, or a combination thereof, and wherein $X_4$ comprises 0 to 6 substituents, selected from the group consisting of $C_{1-6}$ alkyl, halogen, $C_{1-6}$ alkyl halogen, and $C_{1-6}$ alkoxy.

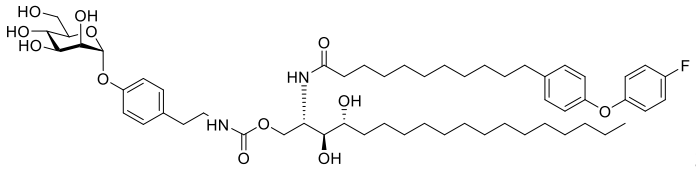
20. The pharmaceutical formulation of claim 19, wherein $X_4$ is $-R_3-O-R_4$, wherein $R_3$ and $R_4$ are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of $C_{1-6}$ alkyl, halogen, $C_{1-6}$ alkyl halogen, and $C_{1-6}$ alkoxy.

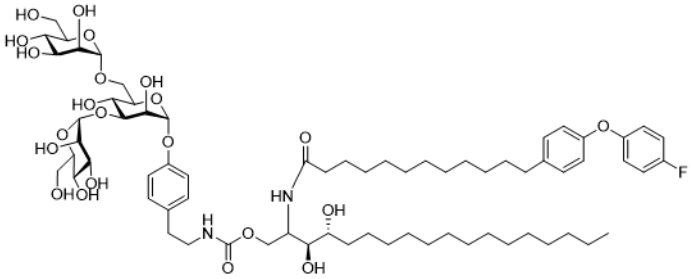
21. The pharmaceutical formulation of any one of claims 1 to 20, wherein $X_4$ is selected from the group consisting of:

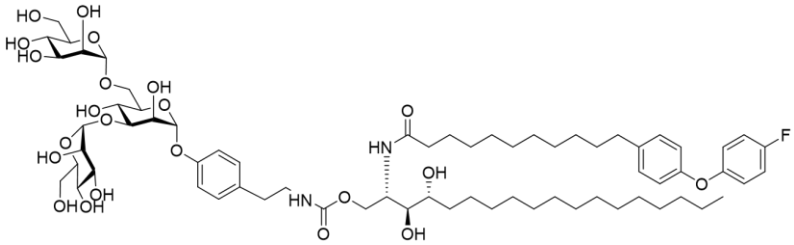


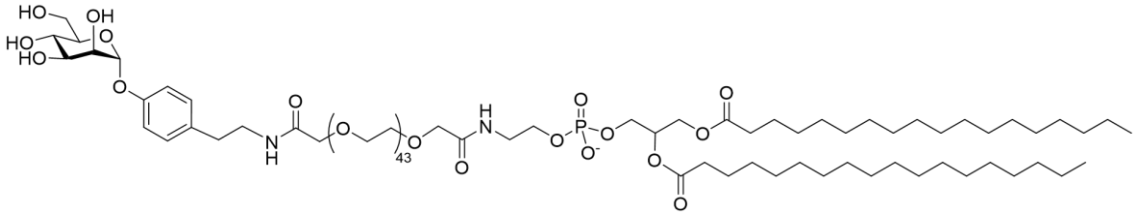

22. The pharmaceutical formulation of any one of claims 1 to 21, wherein the compound is selected from the group consisting of:

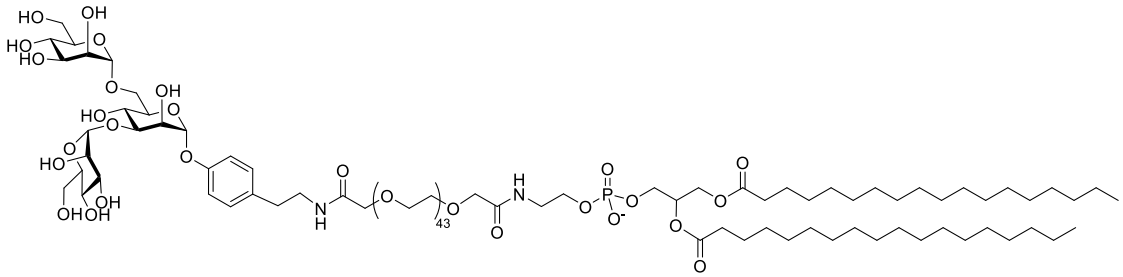


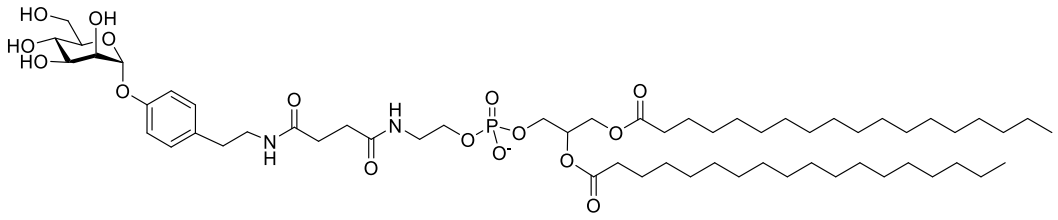

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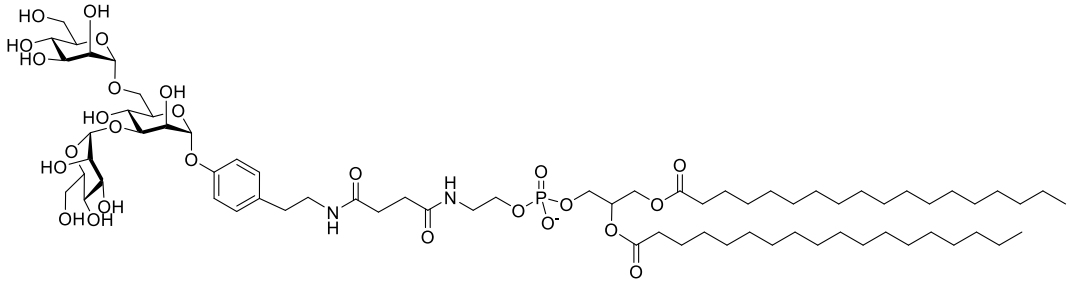

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and


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23. The pharmaceutical formulation of any one of claims 1 to 22, wherein the bi-functional component is not glycolipid C34 or α-galactosylceramide.

24. The pharmaceutical formulation of any one of claims 1 to 23, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof; and optionally

wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxyhexyl)amino]octanoate (SM-102™), (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315™, Pfizer), or a combination thereof; and/or

wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof

25. The pharmaceutical formulation of any one of claims 1 to 24, wherein the excipient comprises a solvent, dispersion media, diluent, dispersion, suspension aid, surface active agent, isotonic agent, thickening or emulsifying agent, preservative, polymer, peptide, protein, cell, hyaluronidase, or mixtures thereof.

27. The pharmaceutical formulation of any one of claims 1 to 26, further comprising an adjuvant, wherein the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, α-galactosylceramide, Aluminum salt, Squalene, MF59, or QS-21. Other examples of adjuvants in some vaccines that can be used in the composition of the present disclosure are aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), mixed aluminum salts, Freund's complete adjuvant, Freund's incomplete adjuvant, AS03 (GlaxoSmithKline), MF59 (Seqirus), and CpG 1018 (Dynavax), or a combination thereof.