(12) STANDARD PATENT APPLICATION (11) Application No. **AU 2026202066 A1**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

(51) International Patent Classification(s)
A61K 9/127 (2025.01)
A61K 31/7032 (2006.01)
A61K 31/7105 (2006.01)
A61K 31/711 (2006.01)
A61K 47/14 (2017.01)
A61K 47/44 (2017.01)
A61P 37/04 (2006.01)

(21) Application No: **2026202066** (22) Date of Filing: **2026.03.18**

(43) Publication Date: **2026.04.09**
(43) Publication Journal Date: **2026.04.09**

(62) Divisional of:
2024252371

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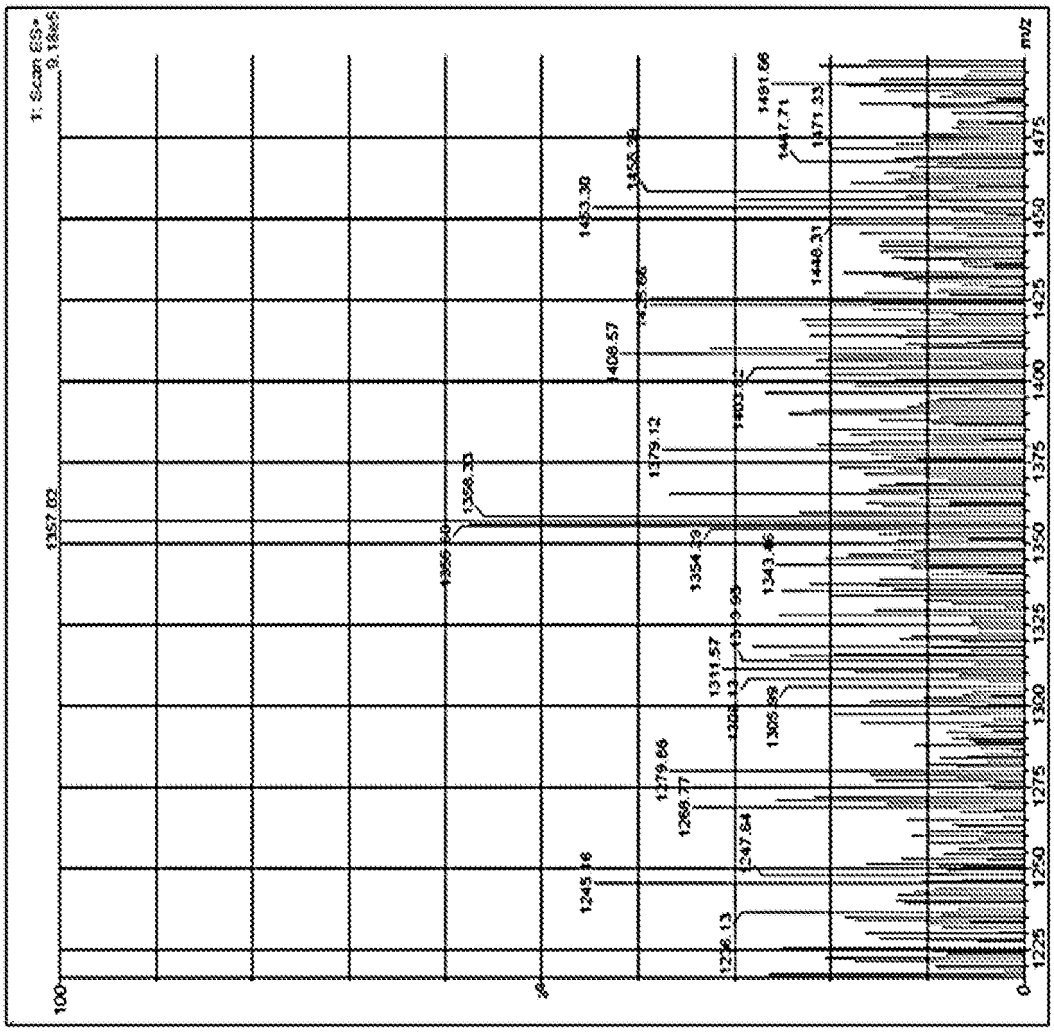
Abstract

The present disclosure relates to lipid nanoparticles (LNP) and kits for making these, for the delivery of therapeutic molecules. Specifically, these LNPs comprise a plural of lipid components, comprising a lipid tail to be incorporated into a bilayer membrane, and a targeting moiety to provide selective delivery of the nanoparticle to a desired tissue.



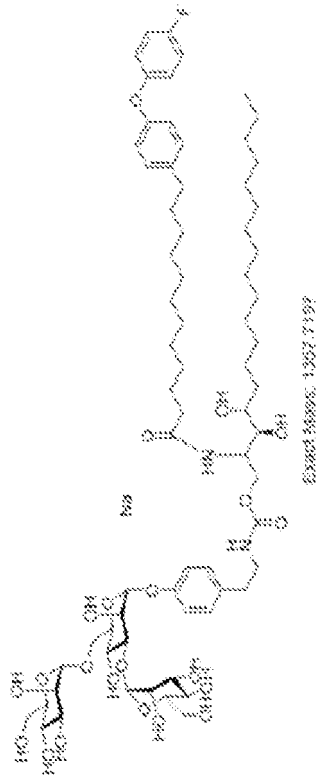


FIG. 12

METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority of U.S. Provisional Patent Applications No. 63/458,102, filed on April 8, 2023, U.S. Provisional Patent Applications No. 63/587,231, filed on October 2, 2023, U.S. Provisional Patent Applications No. 63/575,093, filed on April 5, 2024, and PCT patent publication, filed on April 8, 2024, titled "METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING VACCINES." The present application is a divisional of Australian patent application 2024252371, which is the national phase entry of PCT international application PCT/US2024/023590 (published as WO 2024/215614). The entireties of the aforementioned applications are incorporated herein by reference.

FIELD

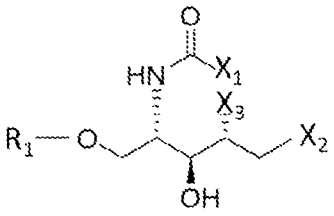
[0002] The instant disclosure relates to novel formulations for targeted nano-delivery. Specifically, the disclosure relates to compositions and methods directed to novel bi-functional nanoparticle formulations capable of selectively delivering a payload to a desired region in a tissue or a specific cell type, including dendritic cells.

BACKGROUND OF THE INVENTION

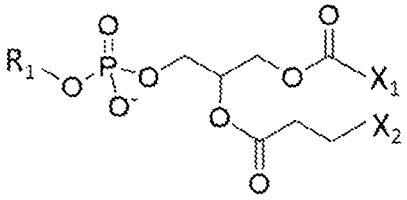
[0003] Localized delivery via nanotechnology has been widely used in scientific, industrial, and clinical applications. It has increasingly become a promising way for drug delivery, providing advantages including improving solubility and penetration of drug molecules. In recent examples of the mRNA vaccines developed against COVID-19 viruses, in view of mRNA molecules' instability and need for low-temperature storage (e.g., −70 °C), lipid nanoparticles (LNP) were developed to encapsulate and stabilize mRNA molecules both in transit and once injected into the human body. The lipid nanoparticles are usually composed of several types of lipids. The ratio of those lipids requires fine-tuning, and the manufacturing of the lipid nanoparticles can be costly, and most importantly, the lipid nanoparticles generally cannot deliver the mRNA molecules with much localized selectivity. Therefore, there is an unmet need for novel nanoparticle formulations with selective delivery functionality.

BRIEF SUMMARY OF THE INVENTION

[0004] One aspect of the present disclosure is directed to a bi-functional compound for forming a lipid nanoparticle suitable for pharmaceutical formulation. The component comprises the formula:



Formula 1; or



Formula 2;

wherein R_1 comprises a substituted or non-substituted glycosyl group;

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.

[0005] One aspect of the present disclosure is directed to a formulation for forming a lipid nanoparticle, comprising the compound of the present disclosure, wherein the compound comprises 1 to 10 mol% of the composition.

[0006] One aspect of the present disclosure is directed to a lipid nanoparticle and/or a lipid nanoparticle formulation. The lipid nanoparticle and/or a lipid nanoparticle formulation comprises a membrane defining an inner space, wherein the membrane is formed with a plurality of lipid components comprising the compound of the present disclosure.

[0007] One aspect of the present disclosure is directed to a bi-functional targeting nanocarrier composition/formulation, which comprises the lipid nanoparticle of the present disclosure.

[0008] One aspect of the present disclosure is directed to a kit and/or a reagent mixture for preparing a lipid nanoparticle, comprising a first reagent, comprising the compound of the present disclosure; and a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.

[0009] One aspect of the present disclosure is directed to a method of targeted payload delivery in a subject in need thereof, comprising administering to the subject an effective amount of the targeting lipid nanoparticle of the present disclosure in a pharmaceutically acceptable formulation, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated by the lipid nanoparticle.

[0010] One aspect of the present disclosure is directed to a method of preventing or treating a disease in a subject in need thereof, comprising administering to the subject an effective amount of the targeting lipid nanoparticle of the present disclosure in a pharmaceutically acceptable formulation, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated within the lipid nanoparticle; and wherein the payload is a therapeutic agent or derives a therapeutic agent.

[0011] One aspect of the present disclosure is directed to a method of boosting an adaptive immune response, comprising administering to the subject an effective amount of the targeting lipid nanoparticle of the present disclosure in a pharmaceutically acceptable formulation, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated within the lipid nanoparticle; and wherein the payload is immunogenic or derives an immunogenic biomolecule.

[0012] In certain embodiments of the bi-functional molecule and formulations thereof, Formula 1 can be structural and/or functional analogs/mimetics with cell-targeting functionalities, and Formula 2 can be structural/functional analogs/mimetics with lipid membrane insertion/anchoring functionalities.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 provides a graphic representation of the FACS analysis results demonstrating the efficacy of the exemplary inventive embodiments. The experiments demonstrated the uptake of the novel nano-delivery formulations targeting BDMCs, according to embodiments of the present disclosure, compared to traditional LNPs. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units (A.U.).

[0014] FIG. 2 provides a graphic representation of the FACS analysis results demonstrating the efficacy of the exemplary novel dendritic cell-targeting formulations of the present disclosure. The experiments showed the uptake of the exemplary novel dendritic targeting formulations of the present disclosure according to embodiments of the present disclosure targeting dendritic cells (DC), B cells, and

T cells, compared with conventional LNPs. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units (A.U.).

[0015] FIG. 3 provides a graphic representation showing the FACS analysis results demonstrating the efficacy of the exemplary novel dendritic targeting formulations of the present disclosure. The experiments showed the uptake of the dendritic cell-targeting formulations according to embodiments of the present disclosure targeting BDMCs compared with conventional LNPs. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units (A.U.). 22-LNP represents the targeting formulation made using compound 22 of the present disclosure, and the percentage in parentheses indicates the molar ratio of compound 22. Likewise, 23-LNP represents the targeting formulation made using compound 23 of the present disclosure, and the percentage in parentheses indicates the molar ratio of compound 23. The negative control was an LNP without using the present disclosure's novel targeting compound/formulation (i.e., "traditional LNP" as described herein).

[0016] FIG. 4 provides a graphic representation showing the FACS analysis results demonstrating the efficacy of the exemplary novel dendritic cell-targeting formulations of the present disclosure. The experiments showed the transfection of the targeting formulations BDMCs compared with LNPs without the compound of the present disclosure. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units (A.U.). 22-LNP represents the targeting formulation made using compound 22 of the present disclosure, and the percentage in parentheses indicates the molar ratio of compound 22. Likewise, 23-LNP represents the targeting formulation made using compound 23 of the present disclosure, and the percentage in parentheses indicates the molar ratio of compound 23. The negative control was an LNP formed without using the present disclosure's novel targeting compound/formulation (i.e., "traditional LNP" as described herein).

[0017] FIG. 5 provides a graphic representation demonstrating the targeting efficacy and specificity of the exemplary formulation based on the distribution of the targeting LNPs in an animal model. The LNPs carried an mRNA configured to encode a luciferase in the targeted cells of the tested animals. The assay would generate detectable luminescence if the LNPs successfully transfect cells and the cells express the luciferase. The results clearly showed tissue-specific targeting of spleen and lymph tissue by the exemplary targeting formulation, thereby providing supporting evidence of immune cell (e.g., dendritic cell) specificity.

[0018] FIG. 6 shows the ^{1}H NMR spectrum of compound 12 of the present disclosure.

[0019] FIG. 7 shows the ^{13}C NMR spectrum of compound 12 of the present disclosure.

[0020] FIG. 8 presents bar charts showing the IFNγ (A) and IL-4 (B) induction by the exemplary targeting LNPs formulation according to an embodiment of the present disclosure *in vivo*. The sera were collected from experimental animals 2 hours, 24 hours, and 48 hours after administration.

[0021] FIG. 9 shows a graphic representation demonstrating the neutralization inhibitory effects of the exemplary LNPs of the present disclosure compared to control LNPs. The neutralization inhibition was evaluated against different dilution factors to show the difference between samples.

[0022] FIG. 10 provides a bar chart demonstrating that the exemplary LNPs according to the present disclosure, carrying mRNA encoding wide-type spike protein, were able to invoke IgG production *in vivo* against wild-type virus and Delta and Omicron strains thereof.

[0023] FIG. 11 shows a comparative bar chart demonstrating the IgG titer (A) and neutralization ability (B) induced respectively by commercially available LNPs vs. LNP formulation formulated based on, and/or constructed with, exemplary compounds according to embodiments of the present disclosure. Both LNPs carried mRNA encoding a wide-type spike protein.

[0024] FIG. 12 shows the LCMS spectrum of compound 21 of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

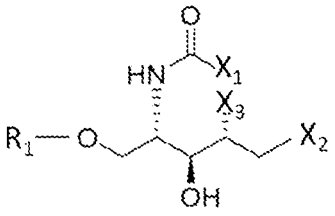
[0025] Nanoparticles have been widely used in various applications. Among them, **lipid nanoparticles (e.g., liposomes)** are the most well-established delivery systems for medicines due to their biocompatibility and biodegradability. A typical liposome has a bilayer structure formed by phospholipids due to their amphipathic properties. The bilayer structure (i.e., a bilayer membrane) encloses an inner space, which can encapsulate hydrophilic molecules, while the bilayer structure itself can carry hydrophobic molecules. Lipid nanoparticles (**LNP**) are also the most studied vehicles for delivering nucleic acids, such as RNA. By encapsulation, LNPs protect nucleic acids from extracellular nucleases, thus allowing for safe delivery to cells.

[0026] LNPs commonly have four kinds of lipid components in desired ratios: (i) helper lipid to encapsulate cargo, (ii) ionizable lipid to enhance endosomal escape and delivery, (iii) cholesterol to promote stability, and (iv) lipid-anchored polyethylene glycol (PEG-lipid) to reduce immune system recognition and improve biodistribution. Cholesterol and PEG-lipid can also be categorized as helper lipids. The properties of a liposome can be adjusted by selecting desired lipid components or ratios thereof. Without wishing to be bound by theories, targeting moieties can also be conjugated with the lipid components to provide selective delivery.

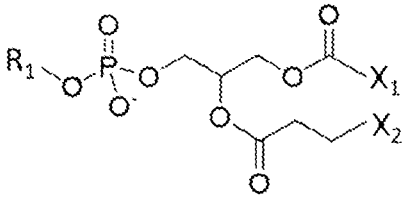
[0027] **TARGETING LIPID COMPOUND**

[0028] One aspect of the present disclosure provides a compound for forming a lipid nanoparticle (LNP) formulation suitable for specific cell targeting. In certain embodiments, the compound is a bi-functional compound containing a glycan-based cell-targeting moiety and a lipid moiety, which can be incorporated into a lipid bi-layer such as an LNP. In some embodiments, the bi-functional compound is designed to have a targeting moiety configured to provide selective delivery functionalities as part of the bi-functional molecule and to have an exemplary lipid tail moiety capable of being incorporated into the lipid bilayer membrane of an exemplary formulation, including a lipid nanoparticle. The compound of the present disclosure can have a dual-tail structure, which comprises two extended structures (e.g., X_1 and X_2 group below). At least one of the two extended structures is configured to be incorporated into a bilayer membrane of the formed lipid nanoparticle. The term "incorporated into a bilayer" herein describes that at least a portion of the extended structure is incorporated into the bilayer. In some embodiments, the entire extended structure is incorporated into the bilayer, but the term is not limited to the scenario.

[0029] In one exemplary aspect, the compound of the present disclosure comprises:



Formula 1; or



Formula 2;

wherein R_1 comprises a substituted or non-substituted glycosyl group; wherein X_1 and X_2 are each independently hydrogen, alkyl, alkenyl, alkynyl, aryloxy, or a substituted version thereof, or $-(CH_2)nX_4$, n is 0 to 50, 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; and wherein X_3 is hydrogen, C_{1-6} alkyl, or hydroxyl.

[0030] ***R_1 group***

[0031] *Targeting Functionality.* In certain embodiments, the R_1 group is configured to provide selective delivery or targeted delivery functionality for the exemplary LNP formulation formed by the component of the present disclosure. In some embodiments, the R_1 group is configured to target an antigen-presenting cell (e.g., a dendritic cell). In some embodiments, the target cell can be other types of immune cells. In yet some other embodiments, the target can be any biological cells where the payload is designed. In certain embodiments, the R_1 group is designed to have a targeting moiety, which can be a ligand of a receipt on a target cell. For example, the R_1 group might be configured to target the DC-SIGN of a dendritic cell.

[0032] Without wishing to be bound by theory, it is believed that mannoside and fucoside can bind a dendritic cell (e.g., via binding to DC-SIGN) with specificity. Therefore, in some embodiments, the R_1 group comprises a mannoside, fucoside, or both as the targeting moiety. The mannoside and/or the fucoside can be a terminal mannose or a terminal fucoside of the R_1 group, which might provide better chances to interact with a dendritic cell.

[0033] In some other embodiments, the R_1 group is configured to target Siglec-1, so the glycosyl group can comprise 9-N-(4H-thieno[3,2-c]chromene-2-carbamoyl)-Neu5Ac-α2,3-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-2, and the glycosyl group can comprise 9-Biphenyl Neu5Ac-α2,6-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-5/E, and the glycosyl group can comprise Neu5Ac-α2,3-Gal-GlcNAc.

[0034] In some embodiments, the R_1 group comprises a formula of R_2-R_A-, wherein R_2 is the substituted or non-substituted glycosyl group, and R_A is an attachment group, and wherein the attachment group is an aryl, an alkyl, an amide, an alkyl amide, a combination thereof, or a covalent bond. In some embodiments, the aryl comprises 0 to 3 substituents (e.g., 1 to 3 substituents), wherein the substituent of the aryl is $C_{1\text{-}6}$ alkyl, halide, or $C_{1\text{-}6}$ alkyl halide. In some embodiments, the attachment group is configured to provide structural flexibilities and/or facilitate the binding between the targeting moiety and the target. In certain embodiments, R_2 is conjugated covalently to R_A at a carbon of the glycosyl group, resulting in an O-glycosylation.

[0035] *Binding in acidic conditions.* In some embodiments, the binding between the glycosyl group of R_1 and a target is Ca^{2+}-correlated, and the calcium coordination might decrease at a low pH environment, resulting in lower binding affinity. Therefore, to provide a better binding affinity under acidic conditions, the attachment group can comprise an aryl group. Without wishing to be bound by any

theories, the aryl group may engage in the CH–π and hydrophobic interactions that enhance the binding under acidic conditions. The aryl group can be an unsubstituted benzene or a benzene substituted with a halide or an alkyl halide (e.g., a CF_3). In some embodiments, the aryl group is coupled with the targeting moiety. For example, the R_1 group can comprise an O-aryl mannoside.

[0036] *Spacer*. In some embodiments, the attachment group of R_1 comprises a spacer. The spacer is configured to provide structural flexibility to R_1. Without wishing to be bound by theories, the flexibility allows the glycosyl group of R_1 to move during the interaction between the targeting moiety and the target, thereby facilitating the binding between them.

[0037] In certain embodiments, a preferred spacer is biocompatible. In some embodiments, the initiator spacer comprises a saturated carbon moiety, a polyethylene glycol (PEG) moiety, or a combination thereof. For example, the spacer can be a polyethylene glycol (PEG) moiety, formed by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18, 20, 24, 30, 36, 40, 48, 50, 55, 60, 65, or 72 (OCH_2CH_2) subunits, or any ranges defined by the foregoing endpoints, such as 2 to 72, 2 to 60, 2 to 48, 2 to 36, 2 to 24, 2 to 18, 2 to 15, 2 to 10, 4 to 72, 4 to 60, 4 to 48, 4 to 36, 4 to 24, 4 to 18, 4 to 15, 4 to 10, 8 to 72, 8 to 60, 8 to 48, 8 to 36, 8 to 24, 8 to 18, 8 to 15, or 8 to 10 (OCH_2CH_2) subunits. In some embodiments, the PEG moiety can be a linear, branched, or star structure.

[0038] *Structural configuration*. In certain embodiments, the glycosyl group can be a linear structure or a branched structure. In some embodiments, the glycosyl group might have a plurality of targeting moieties, for example, 2, 3, 4, 5, 6, 7, 8, 9, or 10 targeting moieties. The plurality of targeting moieties can be arranged in a linear, branched, or star configuration. For example, the glycosyl group might comprise a mono-mannoside, a di-mannoside, or a tri-mannoside, and when the glycosyl group comprises a tri-mannoside, the tri-mannoside can be a linear form or a branched structure, such as a α-1,3-α-1,6-trimannoside. In certain embodiments, it is noticed that a branched configuration (e.g., a tri-mannoside glycan head) shows superior binding affinity to its target receptor.

[0039] In some embodiments, the R_1 group is a substituted glycosyl group. The glycosyl group might comprise 1 to 6 substituents, and each substituent can be $C_{1\text{-}6}$ alkyl, $C_{1\text{-}6}$ alkenyl, halogen, $C_{1\text{-}6}$ alkyl halide, $C_{1\text{-}6}$ alkoxy, amine, nitro, $C_{1\text{-}6}$ alkyl amine, amide, azido, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof. In certain embodiments, the substituent is conjugated to a carbon of the glycosyl group directly or is conjugated to the carbon via an O-yl conjugation (e.g., by replacing the hydrogen of the hydroxyl group on the carbon).

[0040] In certain embodiments, the substituent of the glycosyl group is selected from the group consisting of aryl, 5-membered cycloalkyl, 6-membered cycloalkyl, 5-membered heterocycloalkyl, and 6-membered heterocycloalkyl, and a substituted version thereof, which comprises 1 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halide, C_{1-6} alkoxy, amine, nitro, C_{1-6} alkyl amine, azido, amide, carboxyl, hydroxyl, aryl, cycloalkyl, heterocycloalkyl, or a substituted version thereof, or a combination thereof. In some embodiments, the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.

[0041] In some embodiments, the substituent of the glycosyl group is a substituted or non-substituted aryl, for example, a substituted or non-substituted phenyl group. In certain embodiments, the aryl is substituted with 1 to 6 substituents, each is independently selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halide, C_{1-6} alkoxy, amine, nitro, C_{1-6} alkyl amine, azido, amide, carboxyl, hydroxyl, aryl, cycloalkyl, heterocycloalkyl, or a substituted version thereof, or a combination thereof. In certain embodiments, the substituent of the glycosyl group is a phenyl (benzene ring) substituted with OH, CH_3, NH_2, CF_3, OCH_3, F, Br, Cl, NO_2, N_3, or a combination thereof. For example, the substituted benzene ring can be a phenol group.

[0042] In some embodiments, the R_1 group is a mono-mannoside substituted with 1 to 6 substituents, and each substituent can be C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof. In certain embodiments, the R_1 group is a mono-mannoside substituted with a first substitute and a second substitute; each of the first substitute and the second substitute is independently selected from a group consisting of C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, and sulfite.

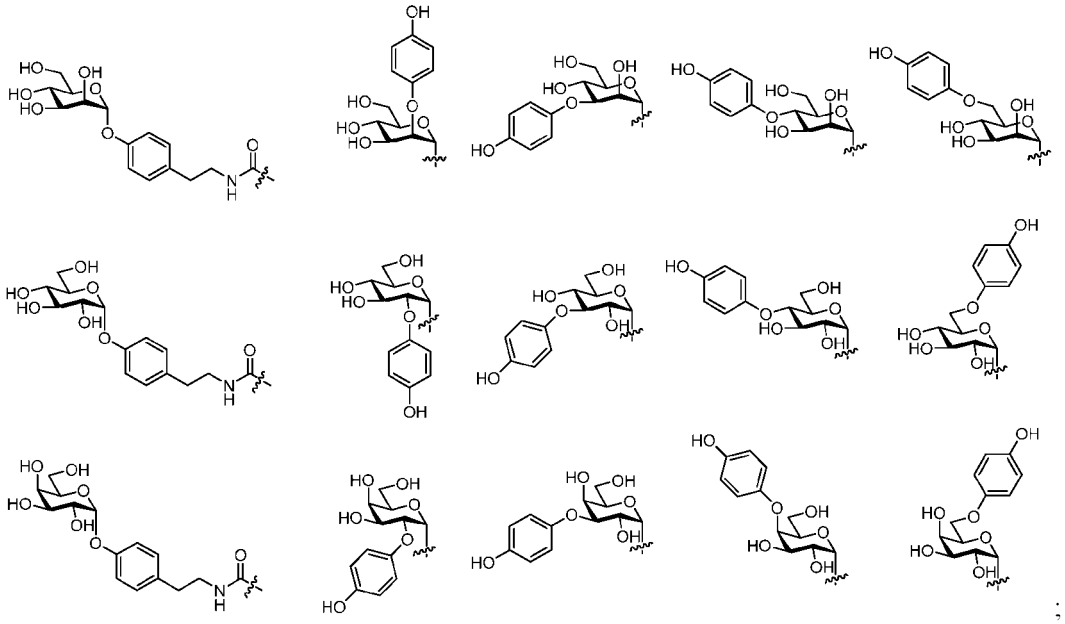
[0043] In some embodiments, the R_1 group comprises a first mannoside and a second mannoside. Each of the first mannoside and the second mannoside is independently substituted with 1 to 6 substituents, and each substituent can be C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

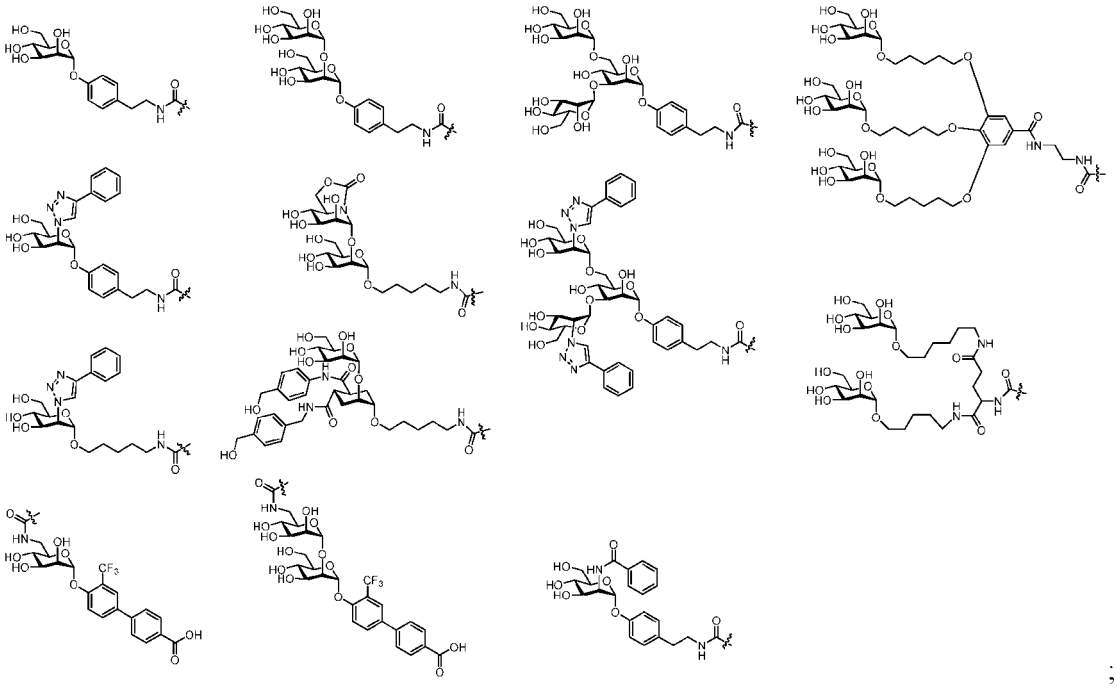
[0044] *Binding affinity*. In some embodiments, the binding affinity between the glycosyl group of R_1 and a target can be defined by a dissociation constant (K_D). In some embodiments, the K_D at pH 7.4 can be 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 3000, 3250, 3500, 3750, 4000, 4250, 4500, 4750, 5000, 5250,

5500, 5750, 6000, 6250, 6500, 6750, 7000, 7250, 7500, 7750, or 8000 nM, or any range defined by the foregoing endpoints, such as, 5 to 8000, 5 to 7000, 5 to 6000, 5 to 5000, 5 to 4000, 5 to 3000, 5 to 2500, 5 to 2000, 5 to 1500, 5 to 1250, 5 to 1000, 5 to 900, 5 to 800, 5 to 700, 5 to 600, 5 to 500, 5 to 400, 5 to 300, 5 to 200, 5 to 150, 5 to 100, 5 to 75, 5 to 50, 5 to 30, 5 to 20, 10 to 8000, 10 to 7000, 10 to 6000, 10 to 5000, 10 to 4000, 10 to 3000, 10 to 2500, 10 to 2000, 10 to 1500, 10 to 1250, 10 to 1000, 10 to 900, 10 to 800, 10 to 700, 10 to 600, 10 to 500, 10 to 400, 10 to 300, 10 to 200, 10 to 150, 10 to 100, 10 to 75, 10 to 50, 10 to 30, or 10 to 20 nM.

[0045] In some other embodiments, the K_D at pH 5 can be 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1250, 1500, 1750, or 2000 nM, or any range defined by the foregoing endpoints, such as, 1 to 2000, 1 to 1500, 1 to 1000, 1 to 900, 1 to 800, 1 to 750, 1 to 700, 1 to 650, 1 to 600, 1 to 550, 1 to 500, 1 to 450, 1 to 400, 1 to 350, 1 to 300, 1 to 250, 1 to 200, 1 to 150, 1 to 100, 1 to 75, 1 to 50, 1 to 40, 1 to 30, 1 to 20, 1 to 10, or to 5, 5 to 2000, 5 to 1500, 5 to 1000, 5 to 900, 5 to 800, 5 to 750, 5 to 700, 5 to 650, 5 to 600, 5 to 550, 5 to 500, 5 to 450, 5 to 400, 5 to 350, 5 to 300, 5 to 250, 5 to 200, 5 to 150, 5 to 100, 5 to 75, 5 to 50, 5 to 40, 5 to 30, 5 to 20, 5 to 10 nM.

[0046] *Examples*. In some embodiments, the R_1 group is selected from the group consisting of (each structure shown below is independent from one another despite whether it is separated using a semicolon with an adjacent structure):





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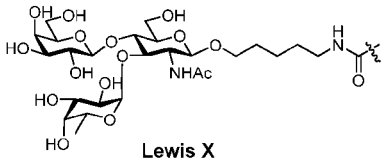
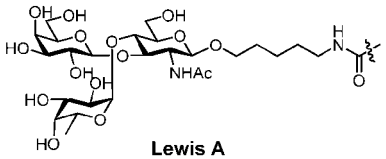
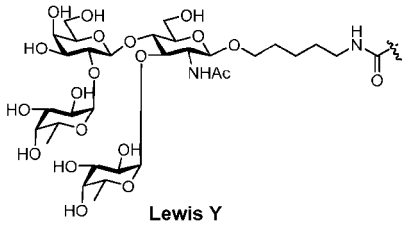


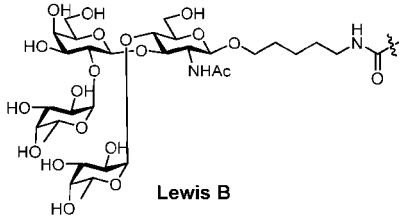
Lewis X



Lewis A



Lewis Y



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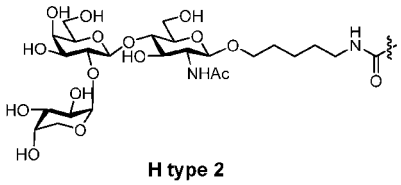
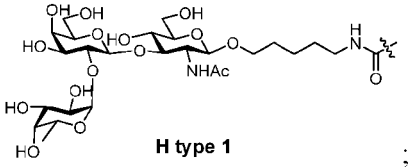
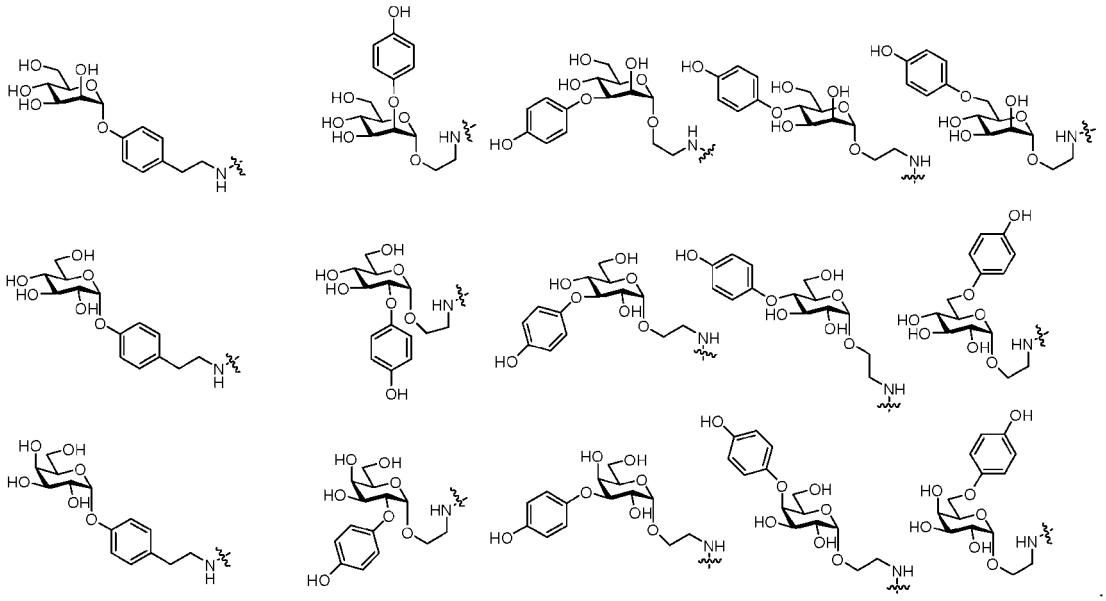


H type 2

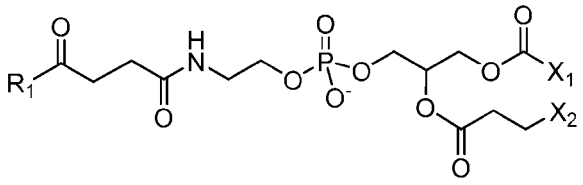


H type 1

; and

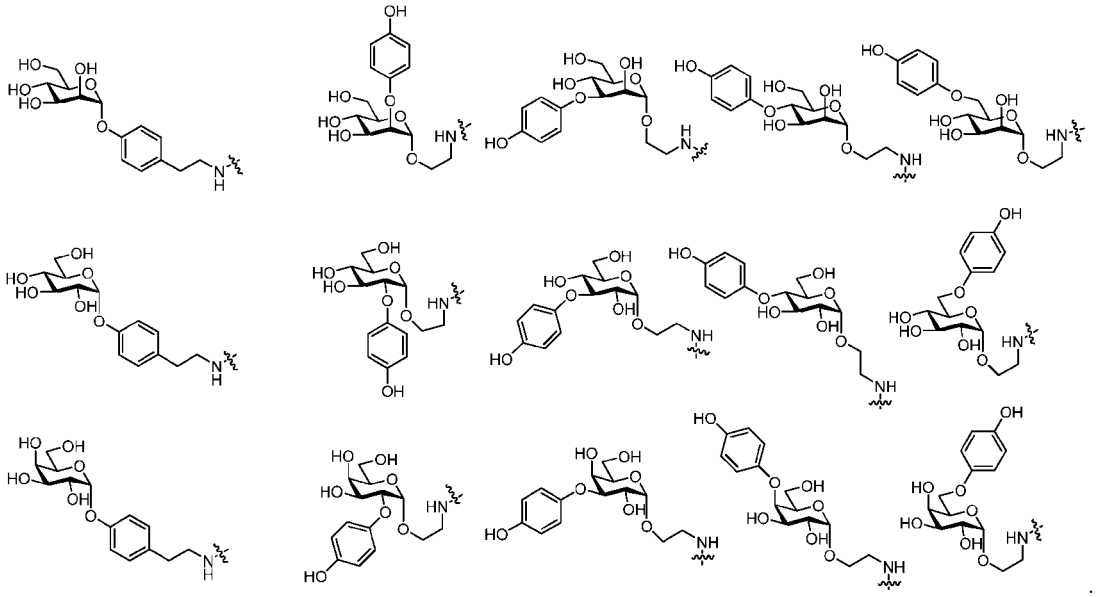


[0047] In some embodiments, the compound of the present disclosure has the structure shown in Formula 3:



Formula 3; and

wherein the R_1 group is selected from the group consisting of (each structure shown below is independent from one another despite whether it is separated using a semicolon with an adjacent structure):



[0048] ***X_1 and X_2***

[0049] The 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. Without wishing to be bound by theories, at least one of the X_1 and X_2 groups is designed to provide the compound of the present disclosure with desired hydrophobicity.

[0050] In some embodiments, at least one of the X_1 and X_2 comprises a saturated hydrocarbon chain, which 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, or any range of carbons defined by the foregoing endpoints, such as 2 to 30, 2 to 28, 2 to 26, 2 to 24, 2 to 20, 2 to 18, 2 to 15, 2 to 12, 2 to 10, 2 to 8, 2 to 6, 2 to 4, 3 to 30, 3 to 28, 3 to 26, 3 to 24, 3 to 20, 3 to 18, 3 to 15, 3 to 14, 3 to 13, 3 to 12, 3 to 11, 3 to 10, 3 to 9, 3 to 8, 3 to 7, 3 to 6, 3 to 5, 4 to 30, 4 to 28, 4 to 26, 4 to 24, 4 to 20, 4 to 18, 4 to 15, 4 to 14, 4 to 13, 4 to 12, 4 to 11, 4 to 10, 4 to 9, 4 to 8, 4 to 7, 4 to 6, 6 to 15, 6 to 14, 6 to 13, 6 to 12, 6 to 11, 6 to 10, 6 to 9, 6 to 8, 10 to 30, 10 to 20, 15 to 30, 15 to 28, 15 to 26, or 15 to 20 carbons.

[0051] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{4-30} alkyl, C_{4-30} alkenyl, C_{4-30} 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.

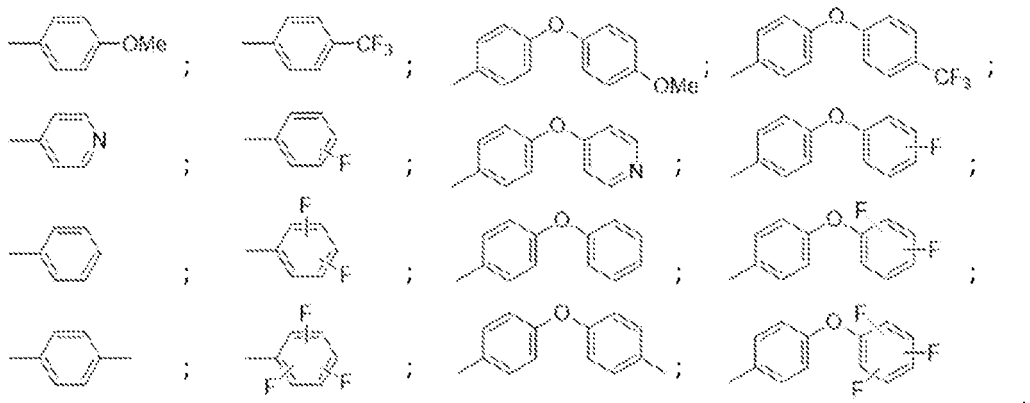
[0052] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{8-30} alkyl, C_{8-30} alkenyl, C_{8-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)nX_4$, n is 8 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided 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.

[0053] In some embodiments, when one of X_1 and X_2 is hydrogen, the other one is not hydrogen. In some embodiments, when one of X_1 and X_2 is hydrogen, the other one comprises a saturated hydrocarbon chain, comprising 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, or any range of carbons defined by the foregoing endpoints, such as 2 to 30, 2 to 28, 2 to 26, 2 to 24, 2 to 20, 2 to 18, 2 to 15, 2 to 12, 2 to 10, 2 to 8, 2 to 6, 2 to 4, 3 to 30, 3 to 28, 3 to 26, 3 to 24, 3 to 20, 3 to 18, 3 to 15, 3 to 14, 3 to 13, 3 to 12, 3 to 11, 3 to 10, 3 to 9, 3 to 8, 3 to 7, 3 to 6, 3 to 5, 4 to 30, 4 to 28, 4 to 26, 4 to 24, 4 to 20, 4 to 18, 4 to 15, 4 to 14, 4 to 13, 4 to 12, 4 to 11, 4 to 10, 4 to 9, 4 to 8, 4 to 7, 4 to 6, 6 to 15, 6 to 14, 6 to 13, 6 to 12, 6 to 11, 6 to 10, 6 to 9, 6 to 8, 10 to 30, 10 to 20, 15 to 30, 15 to 28, 15 to 26, or 15 to 20 carbons. In some embodiments, one of X_1 and X_2 is C_{15-30} alkyl, and the other is $-(CH_2)nX_4$, as defined above.

[0054] In some embodiments, 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. In certain embodiments, X_4 comprises 1 to 3 substituents. The substituent can be, but is not limited to, CH_3, CF_3, F, or OCH_3.

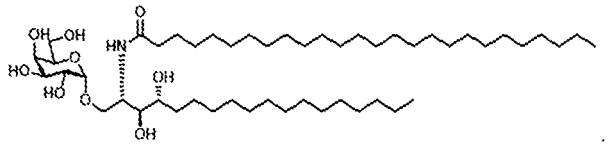
[0055] In some embodiments, X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

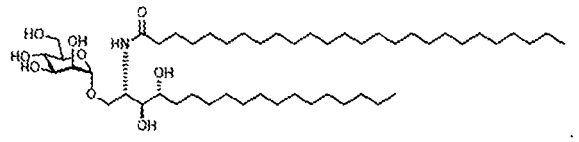
[0056] In certain embodiments, X_4 is selected from the group consisting of:

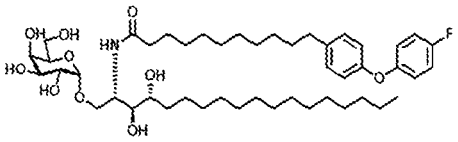


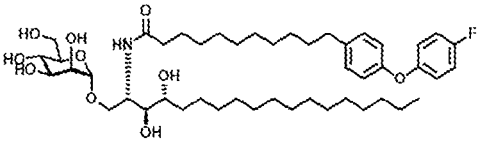
[0057] **Exemplary Compound of the Present Disclosure**

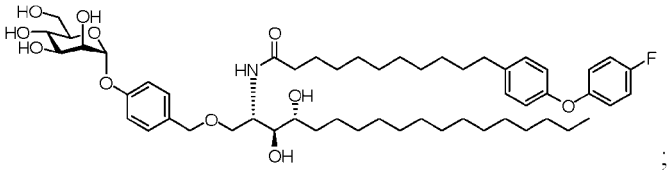
[0058] This section lists some exemplary structures of the compound of the present disclosure. However, the present disclosure is not limited to the exemplary structures listed below or in the specification. In some embodiments, the compound of the present disclosure does not comprise glycolipid C34 or α-galactosylceramide (α-GalCer).







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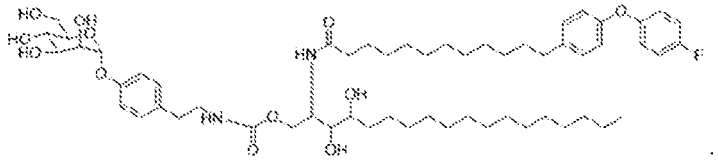
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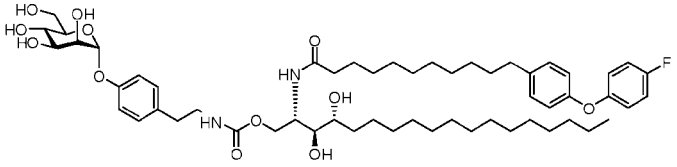
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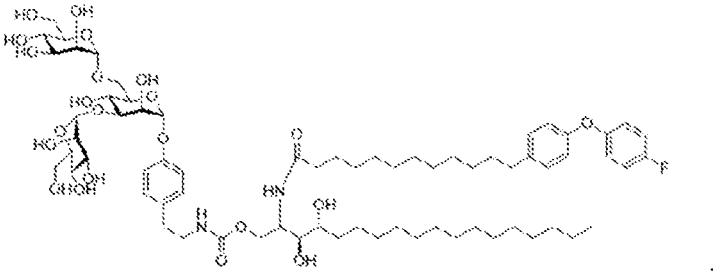
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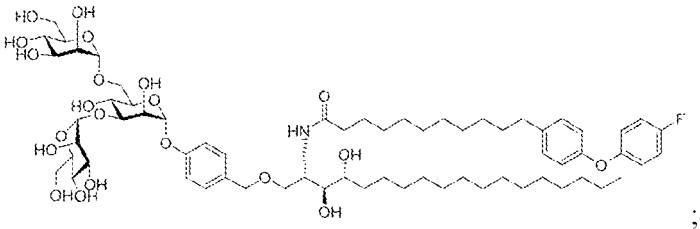
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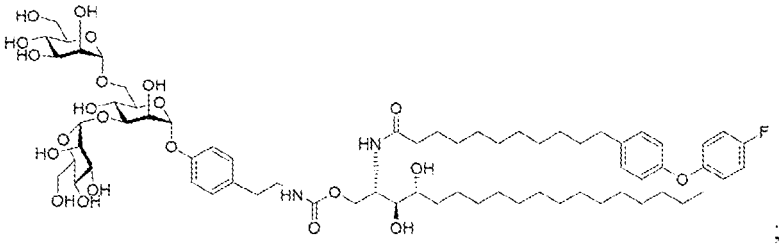
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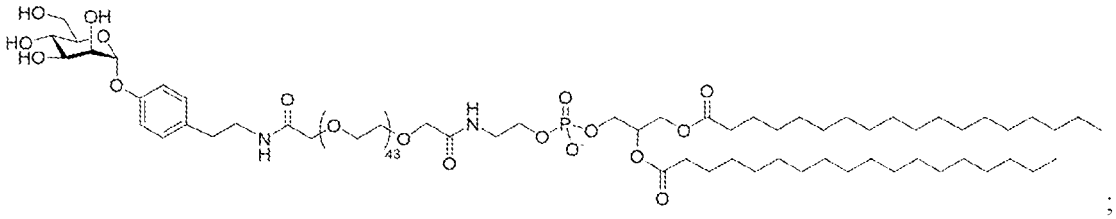

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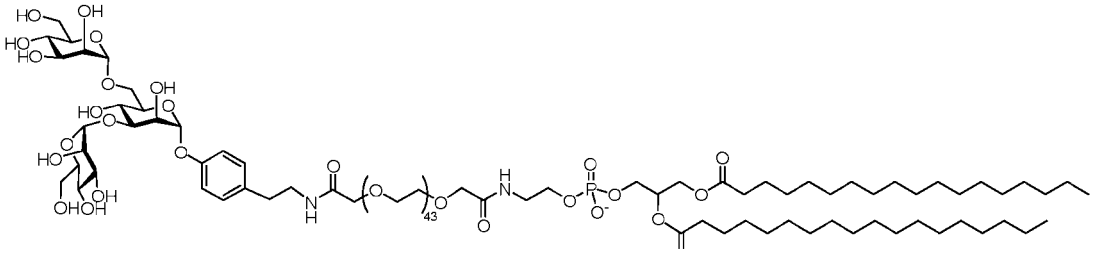

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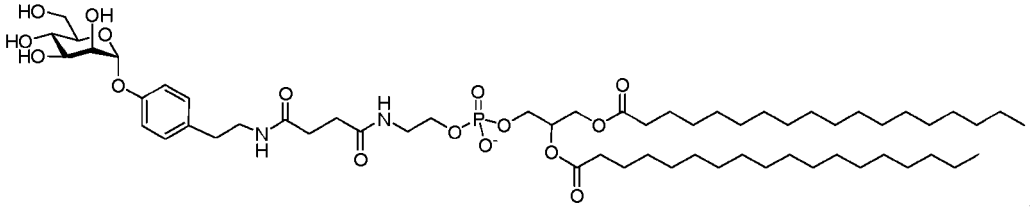

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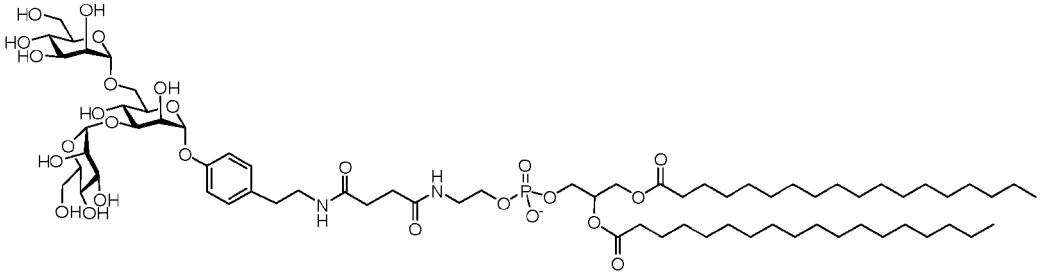

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[0059] **COMPOSITION FOR FORMING A LIPID NANOPARTICLE**

[0060] Another aspect of the present disclosure is directed to a composition, which comprises the compound of the present disclosure. In some embodiments, the compound comprises 1 to 10 mol% of the composition. In certain embodiments, the compound comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 1 to 10 mol%, 1 to 9 mol%, 1 to 8 mol%, 1 to 7 mol%, 1 to 6 mol%, 1 to 5 mol%, 1 to 4 mol%, 1 to 3 mol%, 1 to 2 mol%, 2 to 10 mol%, 2 to 9 mol%, 2 to 8 mol%, 2 to 7 mol%, 2 to 6 mol%, 2 to 5 mol%, 2 to 4 mol%, 2 to 3 mol%, 3 to 10 mol%, 3 to 9 mol%, 3 to 8 mol%, 3 to 7 mol%, 3 to 6 mol%, 3 to 5 mol%, 3 to 4 mol%, 4 to 10 mol%, 4 to 9 mol%, 4 to 8 mol%, 4 to 7 mol%, 4 to 6 mol%, 4 to 5 mol%, 5 to 10 mol%, 5 to 9 mol%, 5 to 8 mol%, 5 to 7 mol%, or 5 to 6 mol% of the composition.

[0061] In some embodiments, the composition might comprise a first compound of the present disclosure and a second compound of the present disclosure. In some embodiments, the composition might comprise more than one, two, or three compounds, each is independently according to the compound of the present disclosure. The first compound, the second compound, or anyone, or any two of the more than one, two, or three compounds can be designed to have the same targeting moiety of the R_1 group, different targeting moiety of the R_1 group targeting the same target, or different targeting moiety of the R_1 group targeting different targets.

[0062] In some embodiments, the composition is configured to form a lipid nanoparticle. The lipid nanoparticle formed is, in some embodiments, expected to perform targeting delivery contributed by the compound of the present disclosure. In some embodiments, the composition configured to form a lipid nanoparticle might further comprise an ionizable lipid, a helper lipid, or a mixture thereof. In some embodiments, the ionizable lipid comprises 30 to 60 mol% of the composition, the helper lipid comprises 5 to 60 mol% of the composition, and the rest percent is a carrier or a solvent.

[0063] In some embodiments, the composition further comprises a payload (i.e., a cargo), which is further discussed below. In certain embodiments, the payload is a first payload, and the composition further encapsulates a second payload. The first payload and the second payload can be the same or different.

[0064] ***Ionizable lipid.***

[0065] In some embodiments, the ionizable lipid would be positively charged at a low pH environment. This feature facilitates the encapsulation of charged molecules, such as nucleic acid (e.g., RNA molecules), and endosomal escape, which allows the release of the cargo/payload into the cytoplasm efficiently. In some embodiments, the ionizable lipid would be neutral at a physiological pH environment, thereby reducing potential toxic effects for a living organism. In some embodiments, the ionizable lipid comprises 30 to 60 mol% of the composition. In certain embodiments, the ionizable lipid comprises about 30, 35, 40, 45, 50, 55, or 60 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 30 to 60 mol%, 30 to 55 mol%, 30 to 50 mol%, 30 to 45 mol%, 30 to 40 mol%, 30 to 35 mol%, 35 to 60 mol%, 35 to 55 mol%, 35 to 50 mol%, 35 to 45 mol%, 35 to 40 mol%, 40 to 60 mol%, 40 to 55 mol%, 40 to 50 mol%, 40 to 45 mol%, 45 to 60 mol%, 45 to 55 mol%, 45 to 50 mol%, or 50 to 60 mol% of the composition. In some embodiments, the ionizable lipid comprises but not limited to 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.

[0066] ***Helper lipid.***

[0067] A helper lipid is a lipid used to increase particle stability and fluidity of lipid nanoparticles. A help lipid can be but is not limited to phosphatidylcholine, cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof. Without wishing to be bound by theories, phosphatidylcholine can help increase the bilayer stability of the lipid nanoparticle and can reduce non-specific binding; cholesterol can fill the gaps between the lipid components forming the lipid nanoparticles and thereby enhancing particle stability by modulating membrane integrity and rigidity; PEGylated lipids (PEG lipids) can enhance colloidal stability and circulation time of lipid nanoparticles in vivo.

[0068] In some embodiments, the helper lipid comprises 5 to 60 mol% of the composition. In certain embodiments, the helper lipid comprises about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 5 to 60 mol%, 5 to 55 mol%, 5 to 50 mol%, 5 to 45 mol%, 5 to 40 mol%, 5 to 35 mol%, 5 to 30 mol%, 5 to 25 mol%, 5 to 20 mol%, 5 to 15 mol%, 5 to 10 mol%, 10 to 60 mol%, 10 to 55 mol%, 10 to 50 mol%, 10 to 45 mol%, 10 to 40 mol%, 10 to 35 mol%, 10 to 30 mol%, 10 to 25 mol%, 10 to 20 mol%, 10 to 15 mol%,15 to 60 mol%, 15 to 55 mol%, 15 to 50 mol%, 15 to 45 mol%, 15 to 40 mol%, 15 to 35 mol%, 15 to 30 mol%, 15 to 25 mol%, 15 to 20 mol%, 20 to 60 mol%, 20 to 55 mol%, 20 to 50 mol%, 20 to 45 mol%, 20 to 40 mol%, 20 to 35 mol%, 20 to 30 mol%, 20 to 25 mol%, 25 to 60 mol%, 25 to 55 mol%, 25 to 50 mol%, 25 to 45 mol%, 25 to 40 mol%, 25 to 35 mol%, 25 to 30 mol%, 30 to 60 mol%, 30 to 55 mol%, 30 to 50 mol%, 30 to 45 mol%, 30 to 40 mol%, 30 to 35 mol%, 40 to 60 mol%, 40 to 55 mol%, 40 to 50 mol%, 40 to 45 mol%, 50 to 55 mol%, or 50 to 60 mol% of the composition.

[0069] *Phosphatidylcholine*. In some embodiments, the phosphatidylcholine comprises 5 to 10 mol% of the composition. In certain embodiments, the phosphatidylcholine comprises about 5, 6, 7, 8, 9, or 10 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 5 to 10 mol%, 5 to 9 mol%, 5 to 8 mol%, 5 to 7 mol%, 5 to 6 mol%, 6 to 10 mol%, 6 to 9 mol%, 6 to 8 mol%, 6 to 7 mol%, 7 to 10 mol%, 7 to 9 mol%, 7 to 8 mol%, 8 to 10 mol%, 8 to 9 mol%, or 9 to 10 mol% of the composition. Examples of phosphatidylcholine include but are not limited to distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0070] *Cholesterol*. In some embodiments, the cholesterol or a derivative thereof comprises 30 to 40 mol% of the composition. In certain embodiments, the cholesterol or a derivative thereof comprises about 30, 32, 34, 36, 38, or 40 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 30 to 40 mol%, 30 to 38 mol%, 30 to 36 mol%, 30 to 34 mol%, 30 to 32 mol%, 32 to 40 mol%, 32 to 38 mol%, 32 to 36 mol%, 32 to 34 mol%, 34 to 40 mol%, 34 to 38 mol%, 34 to 36 mol%, 36 to 40 mol%, 36 to 38 mol%, or 38 to 40 mol% of the composition. Examples of the the cholesterol or a derivative thereof includes but are not limited to cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

[0071] *PEG-lipid*. In some embodiments, the PEG-lipid comprises 1 to 10 mol% of the composition. In certain embodiments, the PEG-lipid comprises about 1, 2, 4, 6, 8, or 10 mol% of the composition, or any range of carbons defined by the foregoing endpoints, such as 1 to 10 mol%, 1 to 8 mol%, 1 to 6 mol%, 1 to 4 mol%, 1 to 2 mol%, 2 to 10 mol%, 2 to 8 mol%, 2 to 6 mol%, 2 to 4 mol%, 4 to 10 mol%, 4 to 8 mol%, 4 to 6 mol%, 6 to 10 mol%, 6 to 8 mol%, or 8 to 10 mol% of the composition. Examples of the PEG-lipid include but are not limited to DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof. In some embodiments, the function of a PEG-lipid in the composition/lipid nanoparticle can be provided by the compound of the present disclosure, especially by embodiments of the present disclosure where the attachment group of R_1 comprises a PEG moiety. In those circumstances, the composition does not need to comprise a PEG-lipid.

[0072] *Examples*. The table below lists some exemplary compositions according to the present disclosure. However, the present disclosure is not limited to the exemplary compositions or those described in the specification.

[0073] Table: Exemplary Embodiments of the Formulation of the Present Disclosure

Embodiments	L1: Ionizable lipid	L2: Phosphatidyl-choline	L3: Cholesterol	L4: PEG-Lipid	L5 the compound of the present disclosure

C1 (control)	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	none
C2-1	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 22 10 mol%
C2-2	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 23 10 mol%
C2-3	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 24 10 mol%
C2-4	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 25 10 mol%
C2-5	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 22 + Compound 23 10 mol%
C2-6	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 24 + Compound 25 10 mol%
C2-7	ALC-0315 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 22 10 mol%
C2-8	ALC-0315 45 mol%	DSPC 9 mol%	Cholesterol 34.65 mol%	DMG-PEG2000 1.35 mol%	Compound 24 10 mol%
C3-1	SM-102 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 5 mol%
C3-2	SM-102 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 23 5 mol%
C3-3	SM-102 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 5 mol%
C3-4	SM-102 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 25 5 mol%
C3-5	ALC-0315 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 5 mol%
C3-6	ALC-0315 47.5 mol%	DSPC 9.5 mol%	Cholesterol 36.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 5 mol%
C4-1	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 20 mol%
C4-2	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 23 20 mol%
C4-3	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 20 mol%

C4-4	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 25 20 mol%
C4-5	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 + Compound 23 20 mol%
C4-6	SM-102 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 + Compound 25 20 mol%
C4-7	ALC-0315 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 20 mol%
C4-8	ALC-0315 40 mol%	DSPC 8 mol%	Cholesterol 30.5 mol%	DMG-PEG2000 1.5 mol%	Compound 23 20 mol%
C5-1	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 10 mol%
C5-2	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.5 mol%	DMG-PEG2000 1.5 mol%	Compound 23 10 mol%
C5-3	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 10 mol%
C5-4	SM-102 45 mol%	DSPC 9 mol%	Cholesterol 34.5 mol%	DMG-PEG2000 1.5 mol%	Compound 25 10 mol%
C6-1	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 10 mol%
C6-2	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 23 10 mol%
C6-3	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 10 mol%
C6-4	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 25 10 mol%
C6-5	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 22 + Compound 23 10 mol%
C6-6	SM-102 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 + Compound 25 10 mol%
C6-7	ALC-0315 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 24 10 mol%
C6-8	ALC-0315 50 mol%	DSPC 10 mol%	Cholesterol 38.5 mol%	DMG-PEG2000 1.5 mol%	Compound 25 10 mol%

[0074] **LIPID NANOPARTICLE (LNP)**

[0075] Another aspect of the present disclosure is related to a lipid nanoparticle (**LNP**). The lipid nanoparticle of the present disclosure comprises a membrane defining an inner space, wherein the membrane is formed with a plurality of lipid components comprising the compound of the present disclosure. The membrane of the lipid nanoparticle can be a bilayer structure, which can be a single bilayer structure or a multiple bilayer structure. In some embodiments, the plurality of lipid components can further comprise an ionizable lipid, a helper lipid, or a combination thereof. The ionizable lipid and the helper lipid can be those described above. Without wishing to be bound by theories, the membrane is formed via hydrophobic interaction between the plurality of the lipid components, while in some circumstances, there might be electrostatic interaction involved in the formation of the membrane. In some embodiments, the plurality of lipid components does not comprise glycolipid C34 or α-galactosylceramide (α-GalCer). In some embodiments, the plurality of lipid compounds comprises glycolipid C34 or α-galactosylceramide (α-GalCer), and the glycolipid C34 or α-galactosylceramide (α-GalCer) comprises less than about 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5% molar ratio of the plurality of lipid compounds, or any range defined by the foregoing endpoints, such as 100% to 5%, 90% to 5%, 70% to 5%, 70% to 5%, 60% to 5%, 50% to 5%, 40% to 5%, 30% to 5%, 25% to 5%, 20% to 5%, 15% to 5%, 10% to 5%, 100% to 10%, 90% to 10%, 70% to 10%, 70% to 10%, 60% to 10%, 50% to 10%, 40% to 10%, 30% to 10%, 25% to 10%, 20% to 10%, or 15% to 10%.

[0076] In some embodiments, the plurality of lipid components of the LNP's membrane might comprise a first compound of the present disclosure and a second compound of the present disclosure. In some embodiments, the plurality of lipid components might comprise more than one, two, or three compounds, each is independently according to the compound of the present disclosure. The first compound, the second compound, or anyone, or any two of the more than one, two, or three compounds can be designed to have the same targeting moiety of the R_1 group, different targeting moiety of the R_1 group targeting the same target, or different targeting moiety of the R_1 group targeting different targets.

[0077] In some embodiments, the compound of the present disclosure comprises at least 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%, of the plurality of lipid components forming the membrane of the LNP, or any range defined by the foregoing endpoints, such as 0.1% to 70%, 0.1% to 60%, 0.1% to 50%, 0.1% to 40%, 0.1% to 35%, 0.1% to 30%, 0.1% to 25%, 0.1% to 20%, 0.1% to 15%, 0.1% to 10%, 0.1% to 5%, 0.1% to 4%, 0.1% to 3%, 0.1% to 2%, 0.1% to 1%, 0.1% to 0.5%, 0.5% to 70%, 0.5% to 60%, 0.5% to 50%, 0.5% to 40%, 0.5% to 35%, 0.5% to 30%, 0.5% to 25%, 0.5% to 20%, 0.5% to 15%, 0.5% to 10%, 0.5% to 5%, 0.5% to 4%, 0.5% to 3%, 0.5% to 2%, 0.5% to 1%, 1% to 70%, 1% to 60%, 1% to 50%, 1% to 40%, 1% to 35%, 1% to 30%, 1% to 25%, 1% to 20%, 1% to 15%, 1% to 10%, 1% to 5%, 1% to 4%, 1% to 3%, 1% to 2%, 5%

to 70%, 5% to 60%, 5% to 50%, 5% to 40%, 5% to 35%, 5% to 30%, 5% to 25%, 5% to 20%, 5% to 15%, 5% to 10%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10 to 15%, 20% to 70%, 20% to 60%, 20% to 50%, 20% to 40%, 20% to 30%, 30% to 70%, 30% to 60%, 30% to 50%, 30% to 40%, 50% to 70%, 50% to 65%, 50% to 60%, 60% to 70%, or 60% to 65%,.

[0078] ***Size of the LNP.***

[0079] In some embodiments, the LNP of the present disclosure has a diameter of 0.01, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 microns, or any range defined by the foregoing endpoints, such as 0.00 to 5, 0.01 to 4, 0.00 to 3, 0.01 to 2, 0.01 to 1, 0.01 to 0.8, 0.01 to 0.6, 0.01 to 0.4, 0.01 to 0.2, 0.01 to 0.1, 0.01 to 0.05, 0.01 to 0.01, 0.05 to 5, 0.05 to 4, 0.05 to 3, 0.05 to 2, 0.05 to 1, 0.05 to 0.8, 0.05 to 0.6, 0.05 to 0.4, 0.05 to 0.2, 0.05 to 0.1, 0.1 to 5, 0.1 to 4, 0.1 to 3, 0.1 to 2, 0.1 to 1, 0.1 to 0.8, 0.1 to 0.6, 0.1 to 0.4, 0.1 to 0.2, 0.5 to 5, 0.5 to 4, 0.5 to 3, 0.5 to 2, 0.5 to 1, 0.5 to 0.8, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 microns. The size of the LNP can be determined by using, but not limited to, Dynamic Light Scattering (DLS). In some embodiments, the LNP of the present disclosure has a polydispersity index (PDI) of about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1, or any range defined by the foregoing endpoints, such as 0.01 to 1, 0.01 to 0.9, 0.01 to 0.8, 0.01 to 0.7, 0.01 to 0.6, 0.01 to 0.5, 0.01 to 0.4, 0.01 to 0.3, 0.01 to 0.2, 0.01 to 0.1, 0.01 to 0.05, 0.1 to 1, 0.1 to 0.9, 0.1 to 0.8, 0.1 to 0.7, 0.1 to 0.6, 0.1 to 0.5, 0.1 to 0.4, 0.1 to 0.3, or 0.1 to 0.2.

[0080] ***Zeta potential and molecular weight.***

[0081] Without wishing to be bound by theories, the zeta potential and molecular weight of an exemplary targeting LNP may affect the cellular uptake of the exemplary targeting LNP. In some embodiments, the exemplary targeting LNP of the present disclosure comprises a zeta potential of about -50, -40, -30, -20, -15, -10, -5, 0, +5, +10, +15, +20, +30, +40, or +50, or any range defined by the foregoing endpoints, such as -50 to +50, -50 to +40, -50 to +30, -50 to +20, -50 to +15, -50 to +10, -50 to +5, -50 to -5, -50 to -10, -50 to -15, -50 to -20, -20 to +50, -20 to +40, -20 to +30, -20 to +20, -20 to +15, -20 to +10, -20 to +5, -20 to -5, -20 to -10, -20 to -15, -15 to +50, -15 to +40, -15 to +30, -15 to +20, -15 to +15, -15 to +10, -15 to +5, -15 to -5, -15 to -10, +5 to +50, +5 to +40, +5 to +30, +5 to +20, +5 to +15, or +5 to +10. In some embodiments, the exemplary targeting LNP of the present disclosure comprises a molecular weight of about 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50 kDa, or any range defined by the foregoing endpoints, such as 1 to 50 kDa, 1 to 40 kDa, 1 to 30 kDa, 1 to 20 kDa, 1 to 15 kDa, 1 to 10 kDa, 1 to 5 kDa, 2 to 50 kDa, 2 to 40 kDa, 2 to 30 kDa, 2 to 20 kDa, 2 to 15 kDa, 2 to 10

kDa, 2 to 5 kDa, 5 to 50 kDa, 5 to 40 kDa, 5 to 30 kDa, 5 to 20 kDa, 5 to 15 kDa, 5 to 10 kDa, 8 to 50 kDa, 8 to 45 kDa, 8 to 40 kDa, 8 to 35 kDa, 8 to 30 kDa, 8 to 25 kDa, 8 to 20 kDa, 8 to 15 kDa, 8 to 10 kDa, 12 to 50 kDa, 12 to 45 kDa, 12 to 35 kDa, 12 to 25 kDa, 12 to 15 kDa, 25 to 50 kDa, 25 to 40 kDa, or 25 to 30 kDa.

[0082] ***Payload***

[0083] In some embodiments, the membrane of the LNP defines an inner space configured to encapsulate or carry a payload (i.e., a cargo). As described herein, "encapsulate a payload" or "carry a payload" refers to the condition that the payload is retained within the LNP by the membrane thereof. The payload can be either contained within the inner space defined by the LNP or embedded within the membrane (e.g., embedded within the bilayer structure). The payload might be able to move freely within the inner space or be attached covalently or non-covalently to the membrane. The encapsulation can be substantial, complete, or partial and does not exclude the possibility that part of the payload might be exposed to the environment outside the LNP. In the embodiments of partial encapsulation, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the payload is retained, enclosed, or surrounded by the membrane of the LNP. In some embodiments, the payload can be a biomolecule such as a nucleic acid, a compound, a polypeptide, a protein, a glycan head, or a combination thereof.

[0084] In some embodiments, the payload is a ribonucleic acid (RNA, e.g., an mRNA) or deoxyribonucleic acid (DNA, e.g., a double-strand DNA or a single-strand DNA), which, after being delivered by using the LNP of the present disclosure to a target cell, can encode a polypeptide or a protein *in vivo*. Nucleic acid, such as a mRNA molecule used in the present disclosure, can be prepared by *in vitro* transcription from a reference nucleic acid. The *in vitro* transcription can be performed as described in the PCT patent publication WO2014/152027, filed on March 13, 2014, which is incorporated by reference in its entirety.

[0085] In some embodiments, the polypeptide or the protein is immunogenic (e.g., antigenic) to an organism to which the exemplary targeting LNP is administered. In such embodiments, the exemplary targeting LNP of the present disclosure is used to encapsulate and carry an immunogenic protein or a nucleic acid configured to encode the immunogenic protein *in vivo*, such as an mRNA molecule in an RNA vaccine. The immunogenic protein can be a protein of a pathogen of viral (e.g., Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV, including SARS-CoV-2), influenza (flu), respiratory syncytial virus (RSV), EBV, DENGUE, VZV, HIV, ZIKA, or NIPAH), bacterial, or fungal origin. In some embodiments, an immunogenic protein can be a spike protein of a virus. In certain embodiments,

the spike protein can be of coronavirus (CoV) origin, such as SARS-CoV, MERS-CoV, and SARS-CoV-2. In some embodiments, examples of the coronavirus (CoV) described herein include but are not limited to, alpha-SARS-CoV2, beta-SARS-CoV2, gamma-SARS-CoV2, delta-SARS-CoV2, omicron-SARS-CoV2, and variants thereof.

[0086] In some embodiments, the payload is a nucleic acid, which can be a polynucleotide having an open reading frame configured to encode a polypeptide or protein *in vivo*. Such a polynucleotide might be modified with a 5'terminal cap, which is generated during an *in vitro*-transcription reaction using the following chemical RNA cap analogs: 3″-O-Me-m7G(5)ppp(5') G [the ARCA cap], G(5)ppp(5')A, G(5')ppp(5')G, m7G(5')ppp(5')A, or m7G(5')ppp(5')G (New England BioLabs, Ipswich, Mass.). A 5'-capping of a modified polynucleotide may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, Mass.). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-O methyl-transferase to generate m7G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-0 methyl-transferase. Enzymes may be derived from a recombinant source. After being transfected into mammalian cells, the modified polynucleotides have a stability of between 12-18 hours, or greater than 18 hours, e.g., 24, 36, 48, 60, 72, or greater than 72 hours.

[0087] In some embodiments, the nucleic acid might be modified. In some embodiments, the nucleic acid might have several (more than one) modifications, the same or different from each other. In some embodiments, the nucleic acid contains, in a particular region, one, two, or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified nucleic acid (e.g., a modified mRNA polynucleotide) exhibits reduced degradation in a cell or organism relative to unmodified ones. In some embodiments, a modified nucleic acid may exhibit reduced immunogenicity in an organism, respectively (e.g., a reduced innate response).

[0088] In some embodiments, the modification can comprise chemical modifications. In some embodiments, the modification can be naturally-occurring, non-naturally-occurring, or both. Some exemplary modifications useful in the present disclosure include but are not limited to, modifications of a sugar, a nucleobase, an internucleoside linkage (e.g., to a linking phosphate, to a phosphodiester linkage, or to the phosphodiester backbone), or a combination thereof. In some embodiments, the nucleic acid (e.g., RNA) used as a payload of the present disclosure can be codon optimized. For example, the nucleic

acid can be modified to enhance the G/C content thereof. The G/C content of a nucleic acid may influence the stability thereof. A nucleic acid having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than those containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides. For example, WO2002/098443 discloses a pharmaceutical composition containing an mRNA stabilized by sequence modifications in the translated region. Due to the degeneracy of the genetic code, the modifications work by substituting existing codons for those that promote greater RNA stability without changing the resulting amino acid.

[0089] In some embodiments, the nucleic acid might further comprise a sequence encoding a signal peptide. The signal peptide might comprise three regions: (1) an N-terminal region of differing length, which usually comprises positively charged amino acids, (2) a hydrophobic region, and (3) a short carboxy-terminal peptide region. In eukaryotes, the signal peptide of a nascent precursor protein (pre-protein) directs the ribosome to the rough endoplasmic reticulum (ER) membrane and initiates the transport of the growing peptide chain across it. A signal peptide usually is not responsible for the final destination of the mature protein, but it is not limited in the present disclosure. Signal peptides are usually cleaved from precursor proteins by an endoplasmic reticulum (ER)-resident signal peptidase. They might remain uncleaved and function as a membrane anchor. In some embodiments, a signal peptide might be designed to fuse with the polypeptide or protein to be encoded by the payload at its C-terminus or N-terminus.

[0090] In some embodiments, the payload can be a therapeutic or prophylactic reagent for treating or preventing a disease (e.g., cancer or an infectious disease). For example, the payload can be an anti-viral agent, including but not limited to ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, remdesivir (GS-5734) and favipiravir (T-705), interferon, adefovir, tenofovir, acyclovir, brivudin, cidofovir, fomivirsen, foscarnet, ganciclovir, amantadine, rimantadine, zanamivir, remdesivir, molnupiravir, and paxlovid. In other examples, the payload can be an anti-cancer agent. In certain embodiments, the payload is a nucleic acid configured to encode a therapeutic or prophylactic reagent.

[0091] In some embodiments, the N/P ratio (positively-chargeable amine (nitrogen atom of the ionizable lipid, N = nitrogen) groups to negatively-charged nucleic acid phosphate (P) groups) of the exemplary targeting LNP encapsulating a nucleic acid is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50, or any range defined by the foregoing endpoints, include or exclude, such as 1 to 50, 1 to 40, 1 to 30, 1 to 20, 1 to 10, 1 to 5, 5 to 50, 5 to 40, 5 to 30, 5 to 20, 5 to 10, 10 to 50, 10 to 40, 10 to 30, 10 to 20, 8 to 40, 8 to 20, 8 to 12, 9 to 50, 9 to

30, or 9 to 15. In another embodiment, the exemplary targeting LNP encapsulating a mRNA has a nanoparticle/mRNA (N/P) ratio of about 10 or about 20.

[0092] In some embodiments where the payload of the LNP is a nucleic acid configured to encode a polypeptide or protein in a target cell, after uptake by the target cell, the LNP is configured to encode *in vivo* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 copies of the polypeptide or protein, or any range defined by the foregoing endpoints, include or exclude, such as, 1 to 50, 1 to 40, 1 to 30, 1 to 20, 1 to 10, 1 to 5, 2 to 50, 2 to 40, 2 to 30, 2 to 20, 2 to 15, 2 to 10, 2 to 8, 2 to 6, 2 to 5, 2 to 4, 5 to 50, 5 to 40, 5 to 30, 5 to 20, 5 to 15, 5 to 10, 5 to 8, 4 to 50, 4 to 45, 4 to 35, 4 to 25, 4 to 15, 4 to 9, 4 to 6, 7 to 50, 7 to 45, 7 to 35, 7 to 25, 7 to 15, or 7 to 9 copies. In some embodiments, after uptake by the target cell, the LNP is configured to encode *in vivo* the polypeptide or protein continually and instantly until the nucleic acid (i.e., the payload) is deactivated *in vivo*.

[0093] ***Kit for Preparing a Lipid Nanoparticle.***

[0094] One aspect of the present disclosure is directed to a kit/reagent mixture for preparing a targeting LNP formulation of the present disclosure. The kit comprises a first reagent and a second reagent, wherein the first reagent comprises the compound of the present disclosure, and the second reagent comprises an ionizable lipid, a helper lipid, or a mixture thereof. The ionizable lipid and the helper lipid can be those described herein. In some embodiments, the second reagent comprises the ionizable lipid, and in some embodiments, the kit further comprises a third reagent comprising the helper lipid. In some embodiments, the kit further comprises a fourth reagent comprising a payload, wherein the payload can be as those described herein.

[0095] *Packaging*. All the components of the kit of the present disclosure can be packaged in a physical container, respectively. In some embodiments, the first reagent and the second reagent are contained in the same container; in other words, in a ready-to-use package. In some other embodiments, the first reagent and the second reagent are contained in separate containers, so a user can decide whether and when to mix the first reagent and the second reagent.

[0096] **COMPOSITIONS/FORMULATIONS**

[0097] One aspect of the present disclosure is directed to a composition comprising the LNP of the present disclosure. The LNP of the composition might encapsulate a payload and is configured to deliver the payload to a target region of an organism. The payload can be as described herein, comprising a nucleic acid, a compound, a peptide, a protein, a glycan head, or a combination thereof. In some

embodiments, the payload can be an immunogenic protein or a nucleic acid configured to encode the immunogenic protein in vivo. In some embodiments, the formulation further comprises a pharmaceutically acceptable excipient, adjuvant, or a combination thereof. In certain embodiments, the composition is a pharmaceutical composition or pharmaceutical formulation.

[0098] In some embodiments, the composition comprises 0.01, 0.05, 0.1, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 95% (w/w) the LNP of the present disclosure, which encapsulates or does not encapsulate a payload, or any range defined by the foregoing endpoints, such as, included or excluded, 0.01% to 95% (w/w), 0.01% to 90% (w/w), 0.01% to 80% (w/w), 0.01% to 70% (w/w), 0.01% to 60% (w/w), 0.01% to 50% (w/w), 0.01% to 40% (w/w), 0.01% to 30% (w/w), 0.01% to 20% (w/w), 0.01% to 10% (w/w), 0.01% to 5% (w/w), 0.01% to 1% (w/w), 0.01% to 0.1% (w/w), 0.1% to 95% (w/w), 0.1% to 90% (w/w), 0.1% to 80% (w/w), 0.1% to 70% (w/w), 0.1% to 60% (w/w), 0.1% to 50% (w/w), 0.1% to 40% (w/w), 0.1% to 30% (w/w), 0.1% to 20% (w/w), 0.1% to 10% (w/w), 0.1% to 5% (w/w), 0.1% to 1% (w/w), 1% to 95% (w/w), 1% to 90% (w/w), 1% to 80% (w/w), 1% to 70% (w/w), 1% to 60% (w/w), 1% to 50% (w/w), 1% to 40% (w/w), 1% to 30% (w/w), 1% to 20% (w/w), 1% to 10% (w/w), 1% to 5% (w/w), 5% to 95% (w/w), 5% to 90% (w/w), 5% to 80% (w/w), 5% to 70% (w/w), 5% to 60% (w/w), 5% to 50% (w/w), 5% to 40% (w/w), 5% to 30% (w/w), 5% to 20% (w/w), or 5% to 10% (w/w). The rest of the percentages of the composition can be an excipient as described herein.

[0099] In some embodiments, the composition is an mRNA vaccine, wherein the LNP encapsulates an mRNA configured to encode an immunogenic protein in vivo. The immunogenic protein can be a virus spike protein or other antigenic molecule of a pathogen. In certain embodiments, the composition of the present invention can be a COVID-19 mRNA vaccine.

[0100] An exemplary COVID-19 mRNA vaccine, as described herein, can be designed based on an mRNA technology to remove the glycan shields of a coronavirus (e.g., SARS-CoV-2) spike protein for better exposing the conserved regions of the spike protein. The mRNA vaccine of coronavirus spike protein has a deletion of glycosylation sites in the receptor binding domain (RBD) or the subunit 2 (S2) domain to expose highly conserved epitopes and elicit antibodies and CD8 T-cell response with broader protection against the alpha, beta, gamma, delta, omicron and various variants, as compared to the unmodified mRNA. The vaccine can be a Low Sugar Universal Vaccine (LSUV) as described in WO2022/221835 (where the mRNA comprises a sequence selected from SEQ ID NO 1-52), WO2022/221837A2 (where the mRNA comprises a sequence selected from SEQ ID No 1-21) and US20200046826A1 (where the mRNA comprises a sequence selected from SEQ ID No 1-20), which are herein incorporated by reference in their entirety.

[0101] In some embodiments, the composition/formulation of the present invention is configured to treat or prevent a disease (e.g., cancer). In such embodiments, the payload carried by the LNP can be a therapeutic reagent, a prophylactic reagent, or a nucleic acid configured to encode the therapeutic reagent or the prophylactic reagent *in vivo*. For example, the composition can be a personalized cancer vaccine (e.g., melanoma), a KRAS vaccine (KRAS-driven), or a checkpoint vaccine (e.g., PD-1, PDL-1 related).

[0102] In some embodiments, the composition/formulation of the present invention can be administered together with another composition (e.g., a vaccine or a medicine). Examples of another composition can be but are not limited to, influenza (flu) vaccine, adenovirus vaccine, anthrax vaccine, cholera vaccine, diphtheria vaccine, hepatitis A or B vaccine, HPV vaccine, measles vaccine, mumps vaccine, smallpox vaccine, rotavirus vaccine, tuberculosis vaccine, pneumococcal vaccine, and Haemophilus influenzae type b vaccine.

[0103] ***Combination compositions***

[0104] In some embodiments, the composition can be a combination composition (e.g., a combo vaccine) comprising a first LNP encapsulating a first payload and a second LNP encapsulating a second payload. The first LNP and the second LNP can be as the LNPs described herein but are different from each other in terms of the structure or properties of the copolymers thereof. For example, wherein the first LNP and the second LNP are different in size, copolymers forming the membrane thereof, payload encapsulated within the LNP, or a combination thereof.

[0105] For example, the first LNP comprises a glycan head configured to bind DC-SIGN, while the second LNP comprises a glycan head configured to bind CD1d. In another example, the first LNP comprises a glycan configured to target an antigen-presenting cell, while the second LNP comprises a glycan configured to target a cancer cell.

[0106] In some embodiments, the first payload and the second payload are different from each other. For example, the first payload can be a protein or peptide, while the second payload can be a nucleic acid. In certain embodiments, the first payload and the second payload can both be mRNA molecules but encode different proteins. For example, the first payload can be a mRNA configured to encode a spike protein of delta-SARS-CoV2, while the second payload can be a mRNA configured to encode a spike protein of omicron-SARS-CoV2.

[0107] ***Additional components of the composition***

[0108] In some embodiments, the composition of the present disclosure can further comprise an adjuvant and/or a non-active substance, such as a pharmaceutically acceptable excipient. In certain embodiments, the adjuvant can be but is not limited to C34, Gluco-C34, 7DW8-5, C17, C23, C30, α-galactosylceramide (α-GalCer), Aluminum salt (e.g., aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), mixed aluminum salts), Squalene, MF59, QS-21, Freund's complete adjuvant, Freund's incomplete adjuvant, AS03 (GlaxoSmithKline), MF59 (Seqirus), CpG 1018 (Dynavax), or a combination thereof.

[0109] In certain embodiments, the pharmaceutically acceptable excipient might comprise 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. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 22nd Edition, Edited by Allen, Loyd V., Jr, Pharmaceutical Press). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition. Formulation of standard pharmaceutically acceptable excipients may be carried out using routine methods in the pharmaceutical art (See Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, USA.).

[0110] In some embodiments, the composition further comprises a phosphate conjugate. Without wishing to be bound by theories, the phosphate conjugate can increase *in vivo* circulation times and/or increase the targeted delivery of the LNP of the present disclosure. Phosphate conjugates for use with the present disclosure may be made using the methods described in the PCT Publication No. WO2013/033438, filed on August 30, 2012, or U.S. Publication No. US2013/0196948, filed on June 23, 2011, the content of each of which is herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in the PCT Publication No. WO2013/033438, filed on August 30, 2012, herein incorporated by reference in its entirety.

[0111] In some embodiments, the composition further comprises a conjugate to enhance the delivery of the LNP of the present disclosure. Without wishing to be bound by theories, the conjugate selected to be used may inhibit the phagocytic clearance of the LNP in a subject. In some examples, the conjugate may be a human membrane protein CD47 or a "self" peptide derived therefrom (e.g., the "self"

particles described by Rodriguez et al. (Science 2013, 339, 971-975), herein incorporated by reference in its entirety).

[0112] In some embodiments where the payload is an immunogenic agent or a nucleic acid configured to encode the immunogenic agent, the composition further comprises an immunostimulatory agent to enhance the immune response induced by the immunogenic agent. As a non-limiting example, the composition may comprise a Th1 immunostimulatory agent, which may enhance a Th1-based response of the immune system (see PCT Publication No. WO2010/123569 and U.S. Publication No. 2011/0223201, each of which is herein incorporated by reference in its entirety).

[0113] In some embodiments, the composition does not include viral components (e.g., viral capsids, viral enzymes, or other viral proteins, for example, those needed for viral-based replication), nor is it packaged within, encapsulated within, linked to, or otherwise associated with a virus or viral particle.

[0114] **METHODS OF USE**

[0115] One aspect of the present disclosure is directed to methods of using the LNP or the targeting formulation of the present disclosure. Particularly, the methods are conducted to obtain a desired effect, such as targeted delivering a payload, preventing or treating a disease, or boosting an adaptive immune response in a subject. In some embodiments, the subject can be but not limited to an animal or a human, to whom the payload is designed to exhibit its efficacy, to whom the treatment or prevention of disease is needed, or to whom the adaptive immune response thereof is required to be boosted.

[0116] ***Methods of targeted payload delivery in a subject***

[0117] In some embodiments, a method of targeted delivery of a payload in a subject is provided, which comprises administering an effective amount of the LNP to the subject or the targeting formulation of the present disclosure. The LNP and the payload are as described herein, and the payload is encapsulated by the LNP. Without wishing to be bound by any theories, the targeted delivery is fulfilled by the compound of the present disclosure. Particularly, the R1 group of the compound of the present disclosure provides the desired binding affinity/specificity targeting a desired region of the subject via the glycosyl group thereof.

[0118] ***Methods of preventing or treating a disease in a subject***

[0119] In some embodiments, a method of preventing or treating a disease in a subject is provided, which comprises administering an effective amount of the LNP to the subject or the formulation of the

present disclosure. The LNP of this method encapsulates a payload, and the payload is a therapeutic agent or derives a therapeutic agent configured to prevent and/or treat the disease.

[0120] Without wishing to be bound by any theories, the LNP or the formulation of the present disclosure provides targeted delivery via the compound of the present disclosure. Therefore, by using the LNP of the present disclosure to deliver the payload, the efficacy of the payload can be more effectively performed. For example, in the embodiments that the R_1 group of the compound comprises a structure binding specifically to the DC-SIGN on dendritic cells, an antigenic or immunogenic payload can be effectively delivered to the dendritic cells to provoke immune responses for preventing disease of concern. This strategy is beneficial in delivering an antigen or a nucleic acid encoding an antigen of a vaccine. Some other examples include having an R_1 group designed to target cancer cells so that an anti-tumor reagent can be delivered effectively to a cancer microenvironment. This strategy can increase the efficacy of the anti-tumor reagent and reduce the treatment's side effects.

[0121] In some embodiments, the disease is characterized by dysfunctional or aberrant protein or polypeptide activity. For example, the disease is selected from the group consisting of rare diseases, infectious diseases, cancer and proliferative diseases, genetic diseases (e.g., cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases.

[0122] In some embodiments, the disease can be cancer or infectious diseases. In certain embodiments, the disease can be a viral-associated infection, including, but not limited to, human parainfluenza virus 3, respiratory syncytial virus (RSV), cytomegalovirus (CMV), human metapneumovirus (hMPV), or SARS-CoV-2 (COVID-19) associated infections.

[0123] ***Methods of boosting an adaptive immune response***

[0124] In some embodiments, a method of boosting an adaptive immune response is provided, comprising administering an effective amount of the LNP of the present disclosure to a subject. The LNP of this method encapsulates a payload within an inner space defined by a membrane of the LNP, and the payload is a therapeutic agent or derives a therapeutic agent configured to provoke an adaptive immune response in the subject.

[0125] Without wishing to be bound by any theories, the LNP of the present disclosure provides targeted delivery to an immune cell via the compound of the present disclosure. In some embodiments, the R_1 group of the compound comprises a structure that binds to an antigen-presenting cell with the

desired specificity or affinity. For example, the R_1 group might comprise a structure binding specifically to the DC-SIGN on dendritic cells so that the LNP is able to deliver the immunogenic payload specifically to the dendritic cells to facilitate the onset of an adaptive immune response.

[0126] In some embodiments, the boosted adaptive immune response is against a disease, including but not limited to cancer or an infectious disease. The infectious disease, for example, can be a viral-associated infection, including, but not limited to, human parainfluenza virus 3, respiratory syncytial virus (RSV), cytomegalovirus (CMV), human metapneumovirus (hMPV), or SARS-CoV-2 (COVID-19) associated infections.

[0127] ***Administration***

[0128] Regarding the methods of the present disclosure, in some embodiments, the subject is administered with a single dose of the LNP or the targeting formulation of the present disclosure, which encapsulates or does not encapsulate a payload. Yet in some embodiments, the subject is administered with the LNP in an initial dose followed by at least one booster dose, e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more follow-up doses, with an interval of each dose in about, 1, 2, 3, 4, 5, 6, 7 days, about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or any range defined by the foregoing endpoints, such as, included or excluded, 1 to 7 days, 1 to 5 days, 1 to 3 days, 1 to 10 weeks, 1 to 8 weeks, 1 to 6 weeks, 1 to 4 weeks, 1 to 2 weeks, 1 to 12 months, 1 to 8 months, 1 to 6 months, 1 to 4 months, 1 to 2 months, or 6 to 12 months. In certain embodiments, the LNP of the present disclosure encapsulating a payload is administered twice at the same or different doses, and the two administrations are separated by 1 day, 3 days, 5 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 1 year, 1 to 5 days, 1 to 2 weeks, 1 to 3 months, 1 to 6 months, 1 month to 1 year, 3 months to 1 year, or 6 months to 1 year.

[0129] *Administration route*. The LNP or formulation as described herein may be administered by any route. Suitable routes include but are not limited to, oral, nasal, mucosal, submucosal, intravenous, intramuscular, intraperitoneal, subcutaneous, intradermal, transdermal, and buccal routes. Some practical topical applications include but are not limited to, a drop, spray, aerosol, gel, or ointment to the mucosal epithelium of the eye, nose, mouth, anus, or vagina. Other possible routes of administration are by spray, aerosol, or powder application through inhalation via the respiratory tract.

[0130] *Effective amount of administration*. The effective amount described herein refers to the amount sufficient to provide a desired effect. In the embodiments where the purpose of administering the

LNP of the present disclosure is to treat a disease, the effective amount refers to a therapeutically effective amount, while in some other embodiments where the purpose is to prevent a disease, the effective amount refers to a prophylactically effective amount.

[0131] Yet in some other embodiments where the purpose of administering the LNP with payload is to boost an adaptive immune response, the effective amount can be determined as an amount sufficient to induce an antigen-specific immune response in a subject to whom the LNP and payload are administered. The antigen-specific immune response can be characterized by measuring an anti-antigenic polypeptide (i.e., the payload or the product of the payload) antibody titer produced in a subject to whom the LNP and payload are administered. In some embodiments, the measurement can be conducted using an Enzyme-linked immunosorbent assay (ELISA).

[0132] In some embodiments, an antibody titer is used to assess whether a subject has had an infection or to determine whether immunizations are required. In some embodiments, an antibody titer is used to determine the strength of an autoimmune response, to determine whether a booster immunization is needed or whether an immunization has been boosted, to determine whether a previous vaccine was effective, and/or to identify any recent or prior infections.

[0133] The effective amount of the methods of the present disclosure can be determined based on several factors, including but not limited to the conditions of the subjects (age, gender, species, body weight, health status, etc.), the progress of the disease to be treated, the administration route, the dosage and interval of the administration, and the nature of the payload. Regarding the nature of the payload, for example, in embodiments where the LNP of the present disclosure is used to carry an mRNA as in a mRNA vaccine, the effective amount can be determined based on the effective amount of the mRNA required to provoke sufficient immune response in the subject. Accordingly, in some embodiments that the payload is mRNA, the effective amount of the methods of the present disclosure is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 micrograms (μg or ug), or any range defined by the foregoing endpoints, such as, include or exclude, 5 micrograms to 1000 micrograms, 5 micrograms to 900 micrograms, 5 micrograms to 800 micrograms, 5 micrograms to 700 micrograms, 5 micrograms to 600 micrograms, 5 micrograms to 500 micrograms, 5 micrograms to 400 micrograms, 5 micrograms to 300 micrograms, 5 micrograms to 200 micrograms, 5 micrograms to 175 micrograms, 5 micrograms to 150 micrograms, 5 micrograms to 125 micrograms, 5 micrograms to 100 micrograms, 5 micrograms to 90 micrograms, 5 micrograms to 80 micrograms, 5 micrograms to 70 micrograms, 5 micrograms to 60 micrograms, 5 micrograms to 50 micrograms, 5 micrograms to 40 micrograms, 5 micrograms to 30

micrograms, 5 micrograms to 20 micrograms, 5 micrograms to 10 micrograms, 10 micrograms to 1000 micrograms, 10 micrograms to 900 micrograms, 10 micrograms to 800 micrograms, 10 micrograms to 700 micrograms, 10 micrograms to 600 micrograms, 10 micrograms to 500 micrograms, 10 micrograms to 400 micrograms, 10 micrograms to 300 micrograms,10 micrograms to 200 micrograms, 10 micrograms to 175 micrograms, 10 micrograms to 150 micrograms, 10 micrograms to 125 micrograms, 10 micrograms to 100 micrograms, 10 micrograms to 90 micrograms, 10 micrograms to 80 micrograms, 10 micrograms to 70 micrograms, 10 micrograms to 60 micrograms, 10 micrograms to 50 micrograms, 10 micrograms to 40 micrograms, 10 micrograms to 30 micrograms, 10 micrograms to 20 micrograms, 50 micrograms to 1000 micrograms, 50 micrograms to 900 micrograms, 50 micrograms to 800 micrograms, 50 micrograms to 700 micrograms, 50 micrograms to 600 micrograms, 50 micrograms to 500 micrograms, 50 micrograms to 400 micrograms, 50 micrograms to 300 micrograms, 50 micrograms to 200 micrograms, 50 micrograms to 175 micrograms, 50 micrograms to 150 micrograms, 50 micrograms to 125 micrograms, 50 micrograms to 100 micrograms, 50 micrograms to 90 micrograms, 50 micrograms to 80 micrograms, 50 micrograms to 70 micrograms, or 50 micrograms to 60 micrograms. 100 micrograms to 1000 micrograms, 100 micrograms to 900 micrograms, 100 micrograms to 800 micrograms, 100 micrograms to 700 micrograms, 100 micrograms to 600 micrograms, 100 micrograms to 500 micrograms, 100 micrograms to 400 micrograms, 100 micrograms to 300 micrograms, 100 micrograms to 200 micrograms, 100 micrograms to 175 micrograms, 100 micrograms to 150 micrograms, 300 micrograms to 1000 micrograms, 300 micrograms to 900 micrograms, 300 micrograms to 800 micrograms, 300 micrograms to 700 micrograms, 300 micrograms to 600 micrograms, 300 micrograms to 500 micrograms, 300 micrograms to 400 micrograms, 500 micrograms to 1000 micrograms, 500 micrograms to 900 micrograms, 500 micrograms to 800 micrograms, 500 micrograms to 700 micrograms, 500 micrograms to 600 micrograms, 600 micrograms to 800 micrograms, or 700 micrograms to 900 micrograms.

[0134] Nevertheless, given the targeted delivery provided by the LNP of the present disclosure, one can expect that the effective amount required in the methods of the present disclosure might be lower than the effective amount required in other non-targeted delivery methods. For example, the effective amount required in the methods of the present disclosure might be lower than the effective amount required in other non-targeted delivery methods by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95, or 99%, or any range defined by the foregoing endpoints, such as, included or excluded, 1 to 99%, 1 to 95%, 1 to 90%, 1 to 80%, 1 to 70%, 1 to 60%, 1 to 50%, 1 to 40%, 1 to 30%, 1 to 20%, 1 to 10%, 1 to 5%, 5 to 99%, 5 to 95%, 5 to 90%, 5 to 80%, 5 to 70%, 5 to 60%, 5 to 50%, 5 to 40%, 5 to 30%, 5 to 20%, 5 to 10%, 10 to 90%, 10 to 80%, 10 to 70%, 10 to 60%, 10 to 50%, 10 to 40%,

10 to 30%, 10 to 20%, 30 to 99%, 30 to 95%, 30 to 90%, 30 to 80%, 30 to 70%, 30 to 60%, 30 to 50%, 30 to 40%, 50 to 99%, 50 to 95%, 50 to 90%, 50 to 80%, 50 to 70%, 50 to 60%, 70 to 99%, 70 to 95%, 70 to 90%, 70 to 80%, 80 to 99%, 80 to 95%, 80 to 90%, 90 to 99%, or 95 to 99%.

[0135] Furthermore, in some embodiments where the LNP of the present disclosure is used to deliver an antigenic agent or a nucleic acid encoding the antigenic agent to induce antibodies against the antigenic agent, the titer of the antibody induced by the present disclosure, compared with the titer of the antibody induced by non-targeted delivery methods, increase 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 logs, or any range defined by the foregoing endpoints, such as, included or excluded 1 to 10 logs, 1 to 8 logs, 1 to 6 logs, 1 to 4 logs, 2 to 9 logs, 2 to 7 logs, 2 to 5 logs, 3 to 10 logs, 3 to 8 logs, 3 to 5 logs, or 4 to 6 logs.

[0136] In some other embodiments, where the LNP of the present disclosure is used to deliver an antigenic agent or a nucleic acid encoding the antigenic agent to induce an immune response against the antigenic agent, the antibody titer against the antigenic agent induced by the present disclosure, compared with that of non-targeted delivery methods, is 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times higher, or any range defined by the foregoing endpoints, such as, included or excluded, 0.1 to 10, 0.1 to 9, 0.1 to 8, 0.1 to 7, 0.1 to 6, 0.1 to 5, 0.1 to 4, 0.1 to 3, 0.1 to 2, 0.1 to 1, 0.1 to 0.5, 0.5 to 10, 0.5 to 9, 0.5 to 8, 0.5 to 7, 0.5 to 6, 0.5 to 5, 0.5 to 4, 0.5 to 3, 0.5 to 2, 0.5 to 1, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, 3 to 10, 3 to 9, 3 to 8, 3 to 7, 3 to 6, 3 to 5, 3 to 4, 5 to 10, 5 to 9, 5 to 8, 5 to 7, 5 to 6, 7 to 10, 7 to 9, 7 to 8, or 8 to 10.

[0137] Yet in some embodiments where the LNP of the present disclosure is used to deliver an antigenic agent or a nucleic acid encoding the antigenic agent to induce an immune response against the antigenic agent, the immune response is induced 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 days earlier than the immune response induced by non-targeted delivery methods, or any range defined by the foregoing endpoints, such as, included or excluded, 1 to 20, 1 to 18, 1 to 14, 1 to 10, 1 to 6, 2 to 20, 2 to 18, 2 to 14, 2 to 10, 2 to 6, 5 to 20, 5 to 18, 5 to 14, or 5 to 10 days earlier.

[0138] In some embodiments, the LNP, as described herein in the methods of the present disclosure, is administered at a dosage level sufficient to deliver a payload from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or

from about 1 mg/kg to about 25 mg/kg, per subject body weight per day, one or more times a day, to obtain the desired *in vivo* effect.

[0139] **DEFINITION**

[0140] Unless specifically defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of microbiology, tissue culture, molecular biology, chemistry, biochemistry, and recombinant DNA technology, which are within the skill of the art. The materials, methods, and examples are illustrative only and not limiting. The following is presented by way of illustration and is not intended to limit the scope of the disclosure.

[0141] Numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions and results, and so forth, used to describe and claim certain embodiments of the present disclosure are to be understood as being modified in some instances by the term "about." A skilled artisan in the field would understand the meaning of the term "about" in the context of the value that it qualifies. The numerical values presented in some embodiments of the present disclosure may contain certain errors resulting from the standard deviation in their respective testing measurements. For example, the term "about," as used herein, refers to a measurable value such as an amount, a temporal duration, and the like and is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, or ±0.1% from the specified value, as such variations are appropriate.

[0142] As used herein, "substantially" means sufficient to work for the intended purpose. The term "substantially" thus allows for minor, insignificant variations from an absolute or perfect state, dimension, measurement, result, or the like, such as expected by a person of ordinary skill in the field, but that does not appreciably affect overall performance. When used with respect to numerical values or parameters or characteristics expressed as numerical values, "substantially" means within ten percent.

[0143] As used herein, "treat," "treatment," and "treating " refer to an approach for obtaining beneficial or desired results, for example, clinical results. For the purposes of this disclosure, beneficial or desired results may include inhibiting or suppressing the initiation or progression of an infection or a disease; ameliorating, or reducing the development of, symptoms of an infection or disease; or a combination thereof.

[0144] As used herein, "preventing" and "prevention" are used interchangeably with "prophylaxis" and can mean complete prevention of infection or prevention of the development of symptoms of that infection, a delay in the onset of a disease or its symptoms, or a decrease in the severity of a subsequently developed infection or its symptoms.

[0145] As used herein, "glycan" or "glycosyl group refers to a polysaccharide, oligosaccharide, or monosaccharide. Glycans can be monomers or polymers of sugar residues and can be linear or branched. A glycan may include natural sugar residues (e.g., glucose, N-acetylglucosamine, N-acetyl neuraminic acid, galactose, mannose, fucose, hexose, arabinose, ribose, xylose, etc.) and/or modified sugars (e.g., 2'-fluororibose, 2'-deoxyribose, phosphomannose, 6' sulfo N-acetylglucosamine, etc.).

[0146] As used herein, "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, saturated or unsaturated, containing the indicated number of carbon atoms. For example, C_{1-6} indicates that the group may have from 1 to 6 (inclusive) carbon atoms in it. Non-limiting examples include methyl, ethyl, iso-propyl, tert-butyl, n-hexyl. A "heteroalkyl" group is an alkyl group in which at least one carbon of the chain has been replaced by a heteroatom. In some embodiments, the heteroalkyl group has 1 to 20 carbon atoms. The term "alkoxy" is intended to mean the moiety —OR, where R is alkyl. The term "aryloxy" is intended to mean the moiety —OR, where R is aryl.

[0147] As used herein, "alkenyl" refers to a hydrocarbon chain including at least one double bond, which may be a straight chain or branched chain, and containing the indicated number of carbon atoms. For example, C_{2-6} indicates that the group may have from 2 to 6 (inclusive) carbon atoms in it. Non-limiting examples include ethenyl and prop-1-en-2-yl.

[0148] As used herein, "alkynyl" refers to a hydrocarbon chain including at least one triple bond, which may be a straight chain or branched chain, and containing the indicated number of carbon atoms. For example, C_{2-6} indicates that the group may have from 2 to 6 (inclusive) carbon atoms in it. Non-limiting examples include ethynyl and 3,3-dimethylbut-1-yn-1-yl.

[0149] As used herein, "cycloalkyl" refers to a nonaromatic cyclic, bicylic, fused, or spiro hydrocarbon radical having 3 to 10 carbons, such as 3 to 8 carbons, such as 3 to 7 carbons, wherein the cycloalkyl group which may be optionally substituted. Examples of cycloalkyls include five-membered, six-membered, and seven-membered rings. A cycloalkyl can include one or more elements of unsaturation; a cycloalkyl that includes an element of unsaturation is herein also referred to as a "cycloalkenyl". Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0150] As used herein, "heterocycloalkyl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring fused or spiro system radical having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Heterocycloalkyls can also include oxidized ring members, such as —N(O)—, —S(O)—, and —$S(O)_2$—. Examples of heterocycloalkyls include five-membered, six-membered, and seven-membered heterocyclic rings. Examples include piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

[0151] As used herein, "aryl" or "aryl group" refers to a moiety formed by the removal of one or more hydrogen ("H") or deuterium ("D") from an aromatic compound. The aryl group may be a single ring (monocyclic) or have multiple rings (bicyclic, or more) fused together or linked covalently. A "carbocyclic aryl" has only carbon atoms in the aromatic ring(s). A "heteroaryl" is intended to mean an aromatic ring system containing 5 to 14 aromatic ring atoms that may be a single ring, two fused rings or three fused rings wherein at least one aromatic ring atom is a heteroatom selected from, but not limited to, the group consisting of O, S and N. Heteroaryls can also include oxidized ring members, such as —N(O)—, —S(O)—, and —$S(O)_2$—. Examples include furanyl, thienyl, pyrrolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl and the like. Examples also include carbazolyl, quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, triazinyl, indolyl, isoindolyl, indazolyl, indolizinyl, purinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl. phenazinyl, phenothiazinyl, phenoxazinyl, benzoxazolyl, benzothiazolyl, 1H-benzimidazolyl, imidazopyridinyl, benzothienyl, benzofuranyl, isobenzofuran and the like.

[0152] As used herein, "amine" refers to a compound that contains a basic nitrogen atom with a lone pair. The term "amino" refers to the functional group or moiety —NH_2, —NHR, or —NR_2, where R is the same or different at each occurrence and can be an alkyl group or an aryl group.

[0153] As used herein, "halogen" or "halo" refers to fluorine, bromine, chlorine, or iodine. In particular, it typically refers to fluorine or chlorine when attached to an alkyl group and further includes bromine or iodine when on an aryl or heteroaryl group.

[0154] As used herein, the term "haloalkyl" refers to an alkyl as defined herein, which is substituted by one or more halo groups. The haloalkyl can be monohaloalkyl, dihaloalkyl, trihaloalkyl, or

polyhaloalkyl, including perhaloalkyl. A monohaloalkyl can have one chloro or fluoro within the alkyl group. Chloro and fluoro are commonly present as substituents on alkyl or cycloalkyl groups; fluoro, chloro, and bromo are often present on aryl or heteroaryl groups. Dihaloalkyl and polyhaloalkyl groups can have two or more of the same halo atoms or a combination of different halo groups on the alkyl. Typically, the polyhaloalkyl contains up to 12, or 10, or 8, or 6, or 4, or 3, or 2 halo groups. Non-limiting examples of haloalkyl include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, 2,2,2-trifluoroethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl and dichloropropyl. A perhalo-alkyl refers to an alkyl having all hydrogen atoms replaced with halo atoms, e.g., trifluoromethyl.

[0155] As used herein, unless otherwise specified, the term "heteroatom" refers to a nitrogen (N), oxygen (O), or sulfur (S) atom.

[0156] **EXAMPLE**

[0157] **Example 1: Synthesis of exemplary compounds of the present disclosure**

[0158] ***Chemical Materials and Methods***

[0159] For chemical synthesis, all starting materials and commercially obtained reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry solvents. ^{1}H and ^{13}C NMR spectra were recorded on Brucker AV-600 spectrometer, and were referenced to the solvent used ($CDCl_3$ at δ 7.24 and 77.23, CD_3OD at δ 3.31 and 49.2, and D_2O at δ 4.80, and DMSO-d_6 at δ 2.5 and 39.51 for ^{1}H and ^{13}C, respectively). Chemical shifts (δ) are reported in ppm using the following convention: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constants (*J*), with *J* reported in Hz. High-resolution mass spectra were recorded under ESI-TOF mass spectroscopy conditions. Silica gel (E, Merck) was used for flash chromatography. IMPACT™ system (Intein Mediated Purification with Affinity Chitinbinding Tag) was purchased from New England Biolabs. His-tag purification resin was purchased from Roche. HiTrap IMAC column (5 mL) was purchased from GE Healthcare Life Sciences. Gel permeation chromatography (GPC) equipped with Ultimate 3000 liquid chromatography associated with a 101 refractive index detector and Shodex columns was used to analyze the polymeric products using THF as the eluent at 30 °C with 1 mL min^{-1} flow rate. The calibration was based on the narrow linear poly(styrene) Shodex standard (SM-105). The

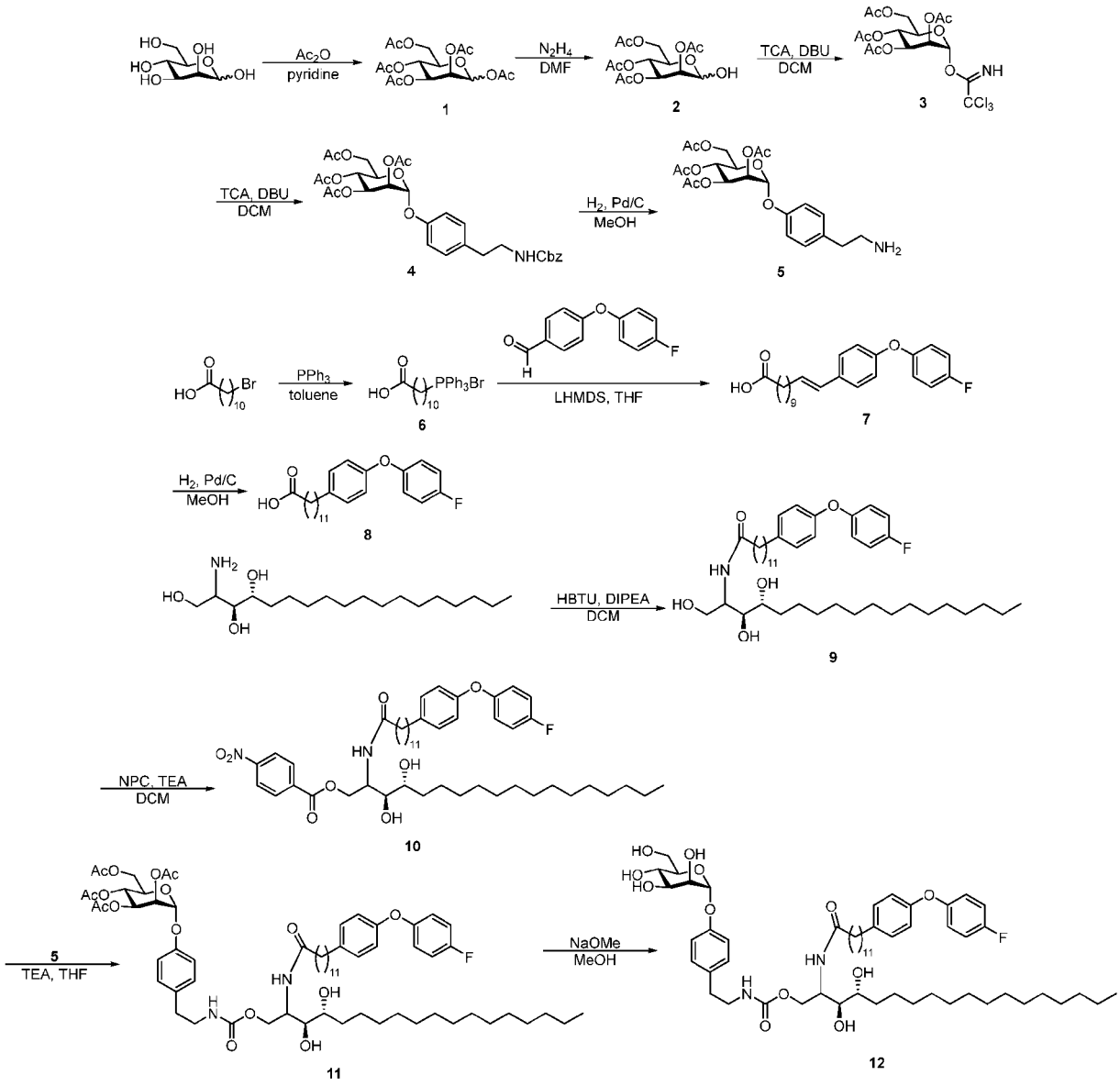
Mw and dispersity of the polymeric products were calculated using DIONEX chromeleon software. Transmission electron microscopy (TEM) images were obtained by a FEI Tecnai G2 F20 S-Twin.

[0160] The chemical materials and methods described herein apply to all examples described in the present disclosure.

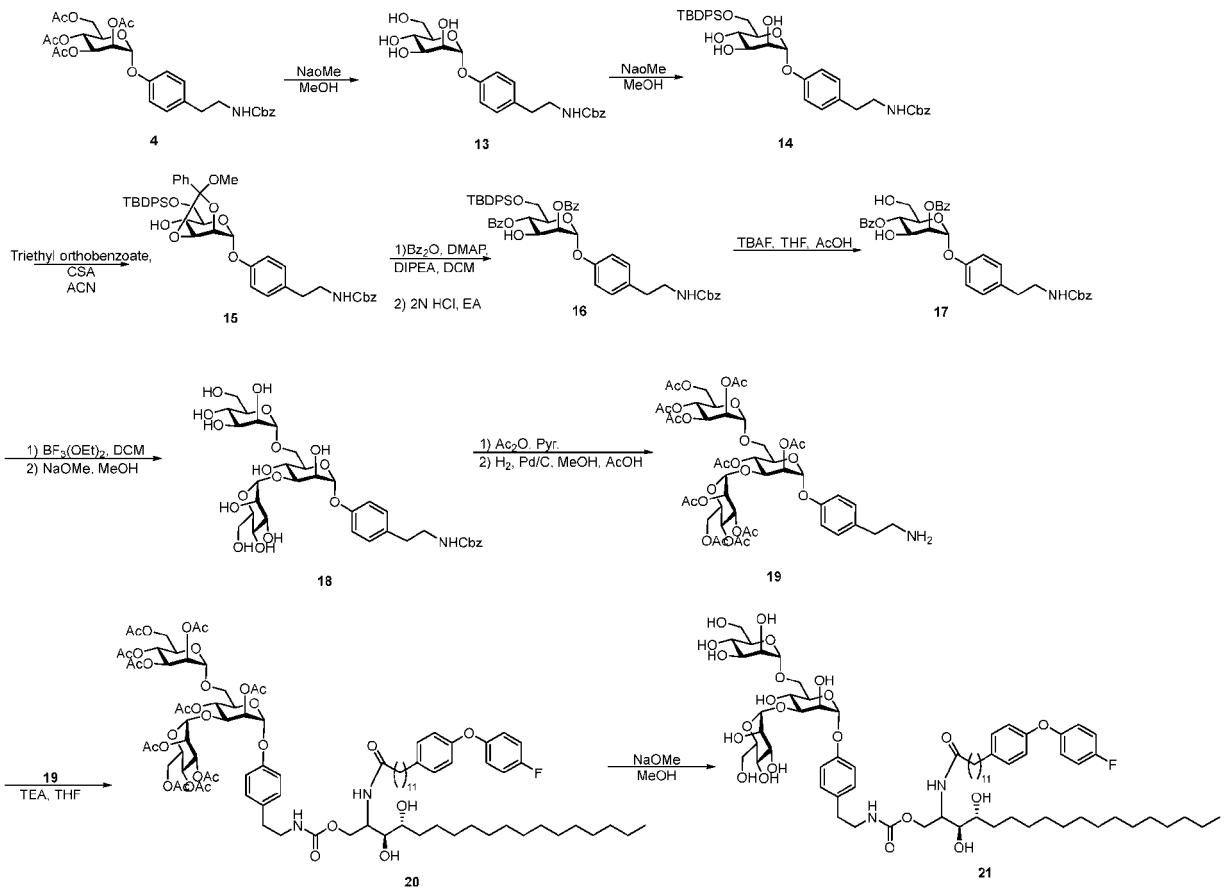
[0161] ***Synthesis and Results***

[0162] The exemplary compounds described here were synthesized according to the synthesis Scheme 1, Scheme 2, and Scheme 3 below. The detailed synthesis procedures are described below.

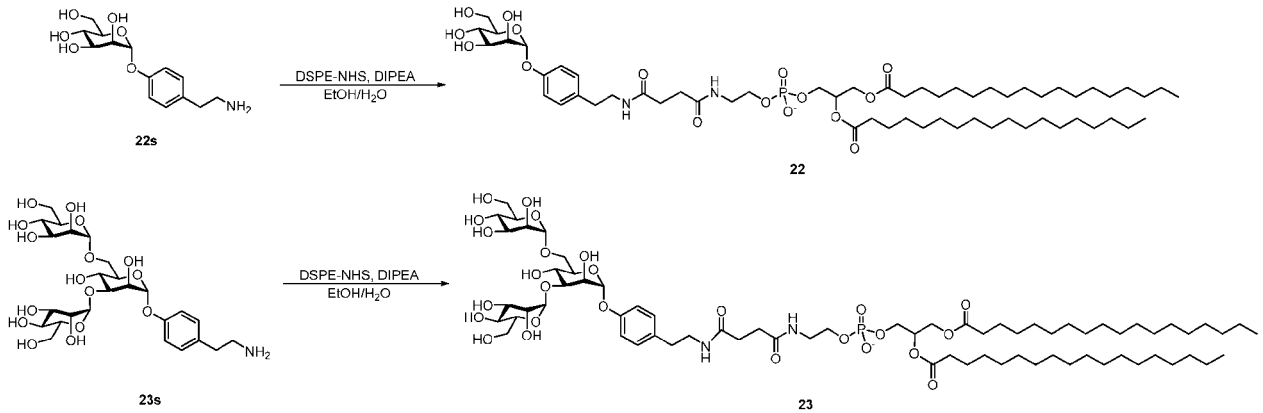
[0163] Scheme 1



[0164] Scheme 2



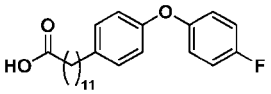
[0165] Scheme 3



[0166] **Compounds 1 to 5**

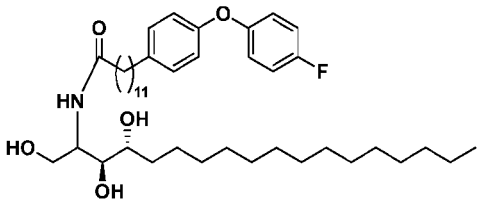
[0167] Compounds **1-5** were synthesized and characterized according to a published protocol (ACS Nano 2021, 15, 309−321).

[0168] **Compound 8**



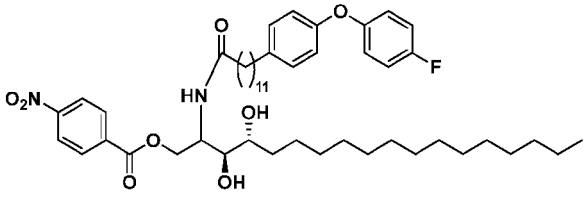
[0169] (11-Carboxynonyl)triphenylphosphonium bromide **6** (2.5 g, 10 mmol) was prepared by refluxing triphenylphosphine (10 mmol) and 11-bromoundecanoic acid (10 mmol). It was then dissolved in 50 ml of tetrahydrofuran (THF) and cooled to 0 °C. lithium bis(trimethylsilyl)amide (LHMDS; 1 M in THF, 20 mmol) was added to the solution to produce an orange ylide. After that, 4-(4-Fluorophenoxy)benzaldehyde (12 mmol) in 20 ml of THF was added dropwise to the solution and stirred for 4 h at room temperature. The reaction was quenched with methanol and concentrated. The residue was extracted with EA and brine and then dried over $MgSO_4$. After removal of the solvent, the mixture was chromatographed on silica gel (EA-Hex =1:2) to give the unsaturated fatty acid **7**. The saturated fatty acid was prepared by catalytic hydrogenation in 50 ml of methanol containing 10 mol% of 10% palladium on charcoal (Pd/C). The reaction mixture was stirred under H_2 at room temperature overnight. The hydrogenated product was filtered through Celite and the resulting solution was concentrated and chromatographed on silica gel (EA-Hex =1:2) to give the product as a yellow solid (66%).

[0170] **Compound 9**



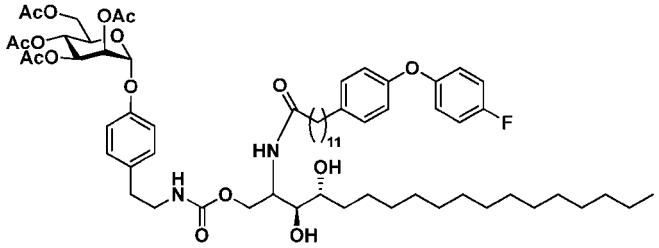
[0171] Compound **9.** Compound **8** (1 mmol) in THF (10 mL) was added EDC (1.5 mmol), HOBt (1.5 mmol), DMAP (0.1 mmol), trimethylamine (2 mmol), and phytosphingosine (1.2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, followed by extraction with EA/H_2O. The collected organic layer was washed with saturated $NaHCO_3$(aq), water and brine, and dried over $MgSO_4$. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1) to yield **9** (74%).

[0172] **Compound 10**



[0173] Compound **9** (1 mmol) in THF (10 mL) was added 4-nitrophenylchloroformate (2 mmol), trimethylamine (2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, and the crude compound was directly used for the next step without further purification.

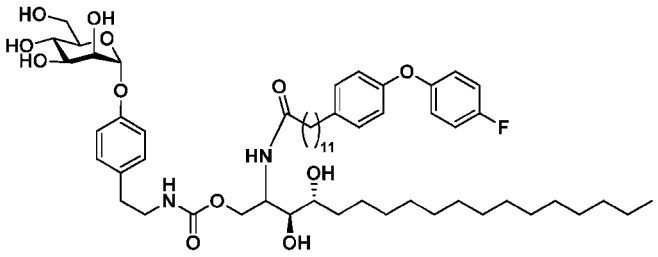
[0174] **Compound 11**



[0175] Compound **5** (1 mmol) in THF (10 mL) was added **10** (1 mmol) and trimethylamine (2 mmol), and the resulting solution was stirred under nitrogen at rt for 2 h. The solvent was then removed by evaporation, followed by extraction with EA/H_2O. The collected organic layer was washed with

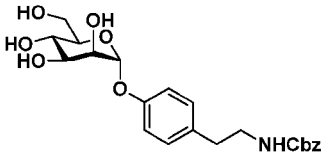
saturated $NaHCO_3$(aq), water and brine, and dried over $MgSO_4$. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **11** (59%).

[0176] **Compound 12**



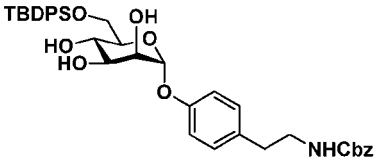
[0177] Compound **11** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at room temperature for 2 hours. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **12** (quant.).

[0178] **Compound 13**



[0179] Compound **4** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **13** (quant.).

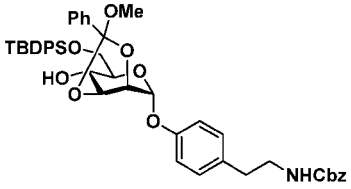
[0180] **Compound 14**



[0181] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo. It was then dissolved in anhydrous DCM (10 mL) and treated with imidazole (1.5 mmol) at 0 °C, followed by the addition of TBDPSCl (1.2 mmol). The mixture was stirred at room temperature for 2.5 h under a nitrogen atmosphere. The reaction was quenched by the addition of

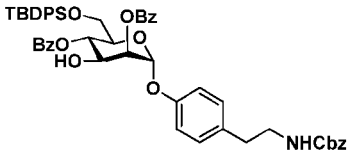
MeOH. After stirring at room temperature for 10 min, the solvent was removed under reduced pressure to give a dry residue that was purified by column chromatography with MeOH/DCM (1/10) to give compound **14** (82%).

[0182] Compound 15



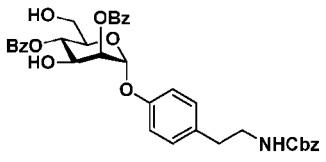
[0183] To a solution of compound **14** (1 mmol) and a catalytic amount of CSA (0.1 mmol) in CH_3CN (20 mL) was added trimethyl orthobenzoate (3 mmol) at room temperature under atmospheric pressure of nitrogen. After stirring for 30 min, Et_3N was added to quench the reaction, and the resulting mixture was dried under reduced pressure. The residue was purified by column chromatography with EA/Hex (1/2) to give compound **15** (79%).

[0184] **Compound 16**



[0185] Compound **15** (1 mmol) was dissolved in DCM (10 mL) and sequentially mixed with DIPEA (2 mmol), benzoic anhydride (2 mmol), and DMAP (0.1 mmol). After stirring for 2 hr, the solvent was evaporated under reduced pressure to give a dry residue and then poured into EA (20 mL) and 2 N HCl (10 mL) with vigorous stirring for 30 min. The solvent was then removed by evaporation, followed by extraction with EA/H_2O. The collected organic layer was washed with ice-cold saturated $NaHCO_3$(aq), water and brine, and dried over $MgSO_4$. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **16** (71%).

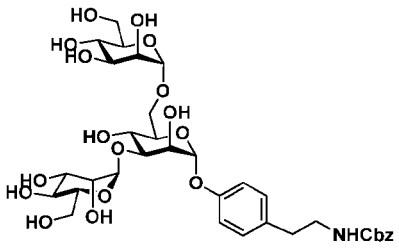
[0186] **Compound 17**



[0187] Compound **16** (1 mmol) was added AcOH (4 mmol) and 1 M TBAF (2.4 mmol in THF) at 0 °C. The resulting mixture was warmed up to room temperature gradually, stirred for another 2 h, and then diluted with EA. The organic layer was washed with saturated $NaHCO_3$(aq), water, and brine, dried with anhydrous $MgSO_4$, and concentrated under reduced pressure. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **47** (88%).

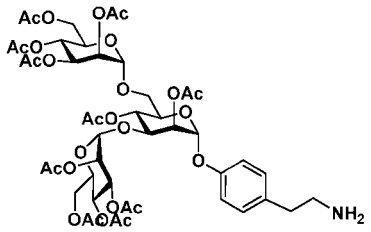
[0188]

[0189] **Compound 18**



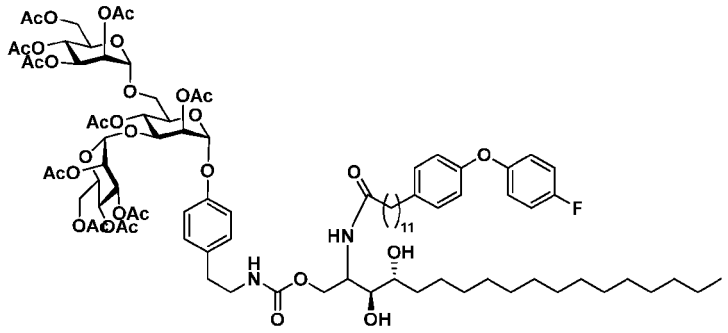
[0190] To a stirred solution of **17** (1 mmol) and 4 A molecular sieve (0.1 g) in anhydrous DCM (10 mL) was cooled to -40°C and then $BF_3(OEt)_2$ (0.1 mmol) was added dropwise to the solution. A solution of **3** in anhydrous DCM was added dropwise to the above mixture and stirred for 1 h at -40°C. After that, the reaction was gradually warmed to room temperature and stirred for another 1 h. The solution was quenched by adding triethylamine, then filtered and added saturated. $NaHCO_3$ aq. and extracted with DCM. The organic layer was dried with $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel to give a trisaccharide product. The product was then dissolved in MeOH, and NaOMe (0.2 eq) was added, and the resulting solution was stirred at room temperature for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo. The deacetylated mixture was purified by Bio-Gel P-2 Gel (Biorad) with H_2O as eluent to obtain a pure trisaccharide. The compound was lyophilized to dryness to give compound **18** (39%).

[0191] **Compound 19**



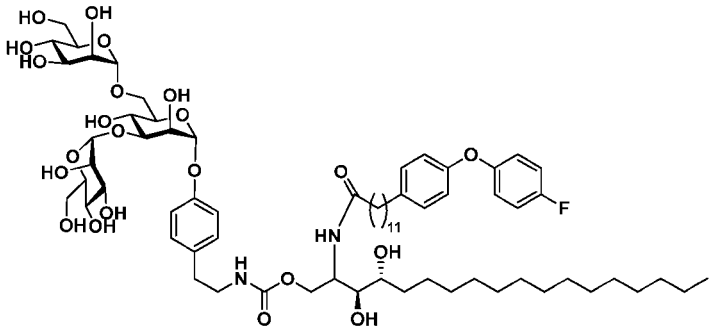
[0192] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo.

[0193] **Compound 20**



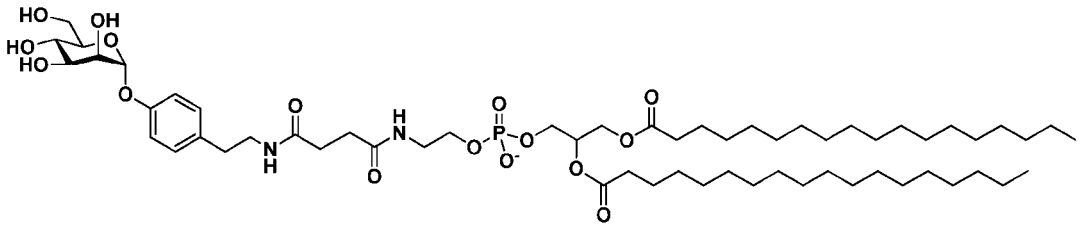
[0194] Compound **19** (1 mmol) in THF (10 mL) was added **10** (1 mmol) and trimethylamine (2 mmol), and the resulting solution was stirred under nitrogen at rt for 2 h. The solvent was then removed by evaporation, followed by extraction with EA/H_2O. The collected organic layer was washed with saturated $NaHCO_3$(aq), water and brine, and dried over $MgSO_4$. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **20**.

[0195] **Compound 21**



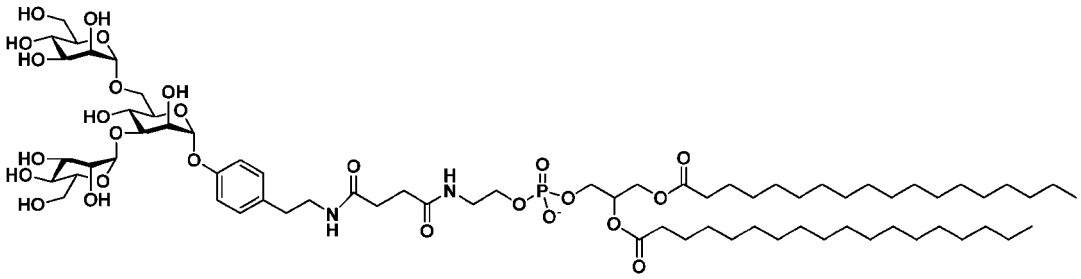
[0196] Compound **20** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at room temperature for 2 hours. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **21** (quant.). The resulting compound 21 was examined using LCMS spectrum, which shows peaks at 1236.13, 1245.16, 1247.64, 1268.77, 1279.86, 1305.99, 1308.13, 1311.57, 1313.93, 1343.46, 1354.29, 1355.60, 1358.33, 1379.12, 1403.82, 1408.57, 1425.66, 1448.31, 1453.30, 1458.39, 1467.71, 1471.33, and 1491.66 (FIG. 12).

[0197] **Compound 22**



[0198] Arylmannoside **22s** (0.1 mmol) in EtOH/H_2O (0.5/0.5 mL) was added to DSPE-NHS (0.1 mmol), and trimethylamine (2 mmol), and the resulting solution was stirred at rt for 12 h. The solvent was removed by evaporation and the crude product was purified Bio-Gel P-2 Gel with H_2O as eluent to yield **22** (79%).

[0199] **Compound 23**



[0200] Aryltrimannoside **23s** (0.1 mmol) in EtOH/H_2O (0.5/0.5 mL) was added DSPE-NHS (0.1 mmol) and trimethylamine (2 mmol), and the resulting solution was stirred at room temperature for 12 h. The solvent was removed by evaporation and the crude product was purified Bio-Gel P-2 Gel with H_2O as eluent to yield **23** (76%).

[0201] **Example 2: Preparation and characterization of the LNP of the present disclosure**

[0202] ***Preparation of LNP***

[0203] A lipid mix solution in EtOH (10 mg/ml) having a molar ratio of 50% SM-102, 10% DSPC, 38.5% cholesterol, and 1.5% DMG-PEG2000 was prepared. An LNP formulation was prepared by mixing the compound of the present disclosure with the lipid mix solution (with a molar ratio of 45% SM-102, 9% DSPC, 34.5% cholesterol, 1.5% DMG-PEG2000, and 10% compound of the present disclosure). The LNP formulation was added into a 1.5 mL tube. Then, a mRNA payload, diluted with citrate buffer before use (10 mM, pH4), was added to the tube at a final concentration of 0.18 ug/uL. The

mRNA aqueous solution in the tube was then quickly added to an ethanol solution and mixed well by vortex for 1 minute. The resulting solution was then dialyzed by micro float-A-Lyzer (8-10 kD) against PBS at 4°C overnight to obtain the LNP of this example. The resulting LNP can be stored at 4°C for a few days before use.

[0204] ***Characterization of LNP***

[0205] *Size Measurement*. The LNP prepared above was examined using dynamic light scattering (DNP) to measure its size. First, 5 μL of the LNP solution was transferred to a clean 1.5 mL tube and diluted with 95 μL of PBS. The mixture was then transferred to a cuvette, and the particle size of the LNP was measured using a Nano ZS machine. The following table shows the sizes and the Polydispersity Index (PDI) of the LNP samples prepared.

[0206] Table: the size and PDI measurement

Sample	Size (nm)_Mean ± SD	PDI_Mean ± SD
Compound 24-LNP	138.5 ± 0.7074	0.1498 ± 0.002694
Compound 25-LNP	161 ± 0.3246	0.1221 ± 0.02095
Compound 12-LNP	177.5 ± 1.79	0.1381 ± 0.02234
Compound 22-LNP	191.7 ± 1.617	0.1625 ± 0.0294
Compound 23-LNP	170.7 ± 1.353	0.1109 ± 0.01918

[0207] *Zeta potential and encapsulation efficiency*. Next, the encapsulation efficiency of the LNP of the present disclosure was evaluated using a Quant-it Ribogreen assay. A 2000-fold diluted quant-it Ribogreen reagent with 1 X TE (working solution) was prepared. Then, an RNA standard dilution series from 0-50 ng/mL (100 μL) was prepared to obtain a standard curve. 5 μL of the LNP solution prepared above was transferred to a clean tube and diluted to a final volume of 100 μL. The working solution of the quant-it Ribogreen reagent (100 μL) was then added to the LNP sample. The fluorescence signal of the sample was then detected using a microplate reader (ex/em 485/535). According to the standard curve, the fluorescence signal was used to calculate the concentration of unencapsulated mRNA in solution (ng/mL). For zeta potential measurement, 0.75 mL DP-intermediate was introduced into capillary cells and measured at 25 °C using Malvern Zetasizer Pro equipment.

[0208] Table: Zeta potential and encapsulation efficiency

Sample	Zeta potential (mV)	Encapsulation efficiency (%)

Compound 24-LNP	0.113	92.15
Compound 25-LNP	-4.382	86.69
Compound 12-LNP	0.932	81.47
Compound 22-LNP	-0.5107	82.86
Compound 23-LNP	0.3875	90.00

[0209] **Example 3: *In vitro* uptake and transfection of mRNA-LNPs in Dendritic cells.**

[0210] ***Experiment 3-1***

[0211] *Splenic cell preparation and BMDC culture.* This example tested the uptake of several exemplary LNPs (as shown in the table below) according to the embodiments of the present disclosure in bone marrow-derived dendritic cells (BMDCs) and splenic cells. To prepare splenic cells, the mouse spleen was homogenized with the frosted end of a glass slide and treated with RBC lysis buffer (Sigma) to deplete red blood cells (RBCs), followed by passing through the cell strainer (BD Biosciences). Bone marrow was isolated from mouse femurs and tibiae and treated with RBC lysis buffer (Sigma-Aldrich) to deplete RBCs. Cells were then cultured in RPMI-1640 containing 10% heat-inactivated FBS (Thermo Fisher Scientific), 1% Penicillin/Streptomycin (Thermo Fisher Scientific), 50 μM 2-mercaptoethanol (Thermo Fisher Scientific), and 20 ng/ml recombinant mouse GM-CSF (eBioscience) at a density of 2×10^5 cells/ml. The cells were supplemented with an equal volume of the complete culture medium (RPMI-1640, 100 U/ml Pen/Strep, 55 μM 2-mercaptoethanol, and 10% FBS) at day 3 and refreshed with one-half the volume of the medium at day 6. On day 8, the suspended cells were harvested.

[0212] Table of the exemplary LNPs tested in this experiment.

Example	L1: Ionizable lipid	L2: Phosphatidyl-choline	L3: Cholesterol	L4: PEG-Lipid	L5 the compound of the present disclosure
1	45 mol%	9 mol%	34.5 mol%	1.5 mol%	Compound 24 10 mol%
2	45 mol%	9 mol%	34.5 mol%	1.5 mol%	Compound 25 10 mol%
3 N.C. (no LNP)	none	none	none	none	none
4 P.C.	50 mol%	10 mol%	38.5 mol%	1.5 mol%	none

[0213] *Treatment of LNPs to splenic cells and BMDCs.* Splenic cells or BMDCs were incubated with different FITC-labeled LNP formulations in RPMI-1640 at 37 ℃ for 1 hour. Cells were blocked with an Fc receptor binding inhibitor (clone: 93, eBioscience) for 20 minutes. Splenocytes were stained with antibodies against CD3 (clone: 17A2, BV421-conjugated, Biolegend), CD19 (clone: 1D3, PECy7-conjugated, BD Biosciences). BMDCs were stained with antibodies against CD11c (clone N418 APC-conjugated, Biolegend). Labeled cells were analyzed using FACSC and Flow Cytometer (BD Biosciences).

[0214] *Flow Cytometry.* After incubation with different mRNA-LNPs, BMDC cells were washed with ice-cold FACS buffer (1% FBS in 1 × DPBS with 0.1% Sodium Azide), and incubated with purified anti-mouse CD16/32 antibody (BioLegend) in FACS buffer on ice for 20 min, followed by washing with FACS buffer. BMDCs were stained with APC anti-mouse CD11c antibody (BioLengend) at 4°C for 30 min, and washed with FACS buffer. Finally, BMDCs were stained with propidium iodide (Sigma-Aldrich). Flow cytometry was performed on a FACS Canto™ flow cytometer (BD Bioscience).

[0215] *Results.* The FACS results are shown in FIG. 1 and FIG. 2 and the table below. FIG. 1 shows that the BMDCs showed specific uptake of the LNPs made using compounds of the present disclosure compared with non-BMDCs. A traditional LNP (i.e., without using the compound of the present disclosure) showed slightly higher uptake by the BMDCs than the non-BDMCs, but the specificity was insignificant compared to that of the LNPs of the present disclosure (56.1/8.62 or 55.2/6.64 vs. 0.48/0.12). Similarly, in FIG. 2, dendritic cells (DCs) showed specific uptake of the LNPs of the present disclosure at least 3 times higher than B cells (30.6/10.7 and 38.3/11.9) and at least 6 times higher than T cells (30.6/0.5 and 38.3/0.68). DCs showed higher uptake of traditional LNPs but the inclination was less significant than that of the LNPs of the present disclosure.

[0216] Table of the uptake results (arbitrary unit of the FITC signals)

Experiment 3-1	L5 the compound of the present disclosure	FITC signal (A.U.)				
		BMDC	Non-BMDC	DCs	B cells	T cells
Example 1	Compound 24	**56.1**	8.62	30.6	10.7	0.50
Example 2	Compound 25	**55.2**	6.64	38.3	11.9	0.68
Example 3	Negative control	0.17	0.18	0.26	0.016	0.022
Example 4	Positive control	0.48	0.12	16.9	1.46	0.26

[0217] ***Experiment 3-2***

[0218] Exemplary LNPs (as shown in the table below) made using different formulations according to the embodiments of the present disclosure were tested in this experiment. Both uptake and transfection were tested to assess whether the payload delivered by the LNPs of the present disclosure can be expressed properly in targeted cells. Bone marrow-derived dendritic cells (BMDCs) were isolated from murine tibia and femurs of 57BL/6 mice. Bone marrow cells were stimulated for 8 days with 20 ng/mL GM-CSF in RPMI medium (RPMI-1640, 100 U/ml Pen/Strep, 55uM 2-mercaptoethanol and 10% FBS). After 8 days of culture, $1x10^6$ BMDCs (centrifuge 400 g, 5mins and replace medium with 1ml Opti-MEM) were plated in 6-well plates, and different samples of LNPs encapsulating mRNA were diluted by 0.25 mL Opti-MEM and incubated with BMDC.

[0219] For *uptake* analysis, FITC-labelled LNPs encapsulating mRNA that encodes a SARS COV2 Spike protein were incubated with the BMDCs at 37°C for 2 hours. For *transfection* analysis, the LNPs encapsulating eGFP mRNA were incubated with the BMDCs at 37°C for 4 hours. After 4 hours of transfection, BMDCs were supplemented with the 1.25ml complete RPMI medium and incubated at 37℃ for 48 hours. The experiments were conducted using FACS, similar to that described above.

[0220] Table of the exemplary LNPs tested in this experiment.

Example	L1: Ionizable lipid	L2: Phosphatidyl-choline	L3: Cholesterol	L4: PEG-Lipid	L5 the compound of the present disclosure
1	47.5 mol%	9.5 mol%	36.5 mol%	1.5 mol%	5 mol% Compound 22
2	45 mol%	9 mol%	34.5 mol%	1.5 mol%	10 mol% Compound 22
3	40 mol%	8 mol%	30.5 mol%	1.5 mol%	20 mol% Compound 22
4	47.5 mol%	9.5 mol%	36.5 mol%	1.5 mol%	5 mol% Compound 23
5	45 mol%	9 mol%	34.5 mol%	1.5 mol%	10 mol% Compound 23
6	40 mol%	8 mol%	30.5 mol%	1.5 mol%	20 mol% Compound 23
7 (control)	50 mol%	10 mol%	38.5 mol%	1.5 mol%	none

[0221] *Results*. The FACS results are shown in FIG. 3 and FIG. 4 and the table below. LNPs made using the compounds of the present disclosure at different molar ratios all showed higher uptake than the negative control ("traditional" LNP without using the compound of the present disclosure). The data also confirms that the LNPs of the present disclosure not only can deliver the payload into the

targeted cells but also can transfect and allow the targeted cells to express the payload. Given the higher specificity towards the targeted cells, the transfection signals detected from the groups using the LNPs of the present disclosures were also significantly higher than those detected from the traditional group. This result suggests that using the LNPs of the present disclosure allows a lower dosage of the payload for a similar outcome.

[0222] Table showing the results of uptake and transfection (arbitrary unit of the FITC signals)

Experiment 3-2	L5 the compound of the present disclosure	FITC signal intensity (A.U.) derived from uptake	FITC signal intensity (A.U.) derived from transfection
Example 1	5 mol% Compound 22	12.0	1.58
Example 2	10 mol% Compound 22	6.11	2.35
Example 3	20 mol% Compound 22	11.0	1.88
Example 4	5 mol% Compound 23	13.4	2.11
Example 5	10 mol% Compound 23	13.6	1.67
Example 6	20 mol% Compound 23	23.6	2.30
Example 7 (control)	None (traditional LNP)	0.38	0.71

[0223] ***Experiment 3-3***

[0224] To assess the binding of DC-SIGN to the LNPs of the present disclosure, ELISA plates were coated with exemplary LNPs in PBS at 4 °C overnight, respectively. The plates were incubated with diluted DC-SIGN ECD (15 to 0.075 nM in HEPES buffer containing 20 mM HEPES, 150 mM NaCl, 10 mM $CaCl_2$, 0.1% BSA) at pH 7.4, 6.0, and 5.0 for 1 hour at room temperature. The bound DC-SIGN ECD was detected using HRP-conjugated anti-DC-SIGN (B2) IgG antibody (Santa Cruz Biotechnology). After 1 hour of incubation at room temperature, the plates were treated with tetramethlybenzidine (TMB) for 10 min. The optical density was measured at 450 nm after adding 0.5 M sulfuric acid to the plates using a microplate reader. The apparent Kd was calculated using a nonlinear regression curve fit for total binding using GraphPad Prism.

[0225] **Example 4:** ***In vivo delivery of luciferase mRNA-LNP***

[0226] This experiment tested the targeted delivery of the LNPs of the present disclosure (shown in the table below) *in vivo*. The LNPs tested in this experiment carried mRNA encoding luciferase. Mice were injected intravenously with the LNPs (200 μL) and maintained for one hour or six hours before *In vivo* Imaging System (IVIS®) measurement. For the IVIS measurement, the animals were first

anesthetized using the rodent anesthesia system with isoflurane (2.5% (vol/vol) in 0.2 L/min O2 flow). Then, the animals were injected intravenously with D-luciferin solution (dissolved in 1X PBS; 150 mg/kg body weight). After 3 minutes from the injection, the animals were scanned using the IVIS imaging system (data not shown). After imaging, the animals were euthanized in a CO_2 chamber. The organs (heart, lungs, liver, spleen, kidneys, and lymph nodes) of the animals were collected, and the luminescence was detected and quantified using the IVIS system.

[0227] Table of the exemplary LNPs tested in this experiment.

Example	L1: Ionizable lipid	L2: Phosphatidyl-choline	L3: Cholesterol	L4: PEG-Lipid	L5 the compound of the present disclosure
1	45 mol%	9 mol%	34.5 mol%	1.5 mol%	10 mol% Compound 22
2	45 mol%	9 mol%	34.5 mol%	1.5 mol%	10 mol% Compound 12
7 (control)	50 mol%	10 mol%	38.5 mol%	1.5 mol%	none

[0228] *Results*. The results (FIG. 5) show that both compound 22-LNP and compound 12-LNP tend to accumulate in spleens and lymph nodes. While compound 12-LNP also accumulated in livers, compound 22-LNP showed high-level specificities targeting spleens and lymph nodes. The results demonstrate the targeting delivery functionalities of the lipid nanoparticle formulations of the present disclosure, which matches the observations of the experiments above.

[0229] **Example 6: Immunization**

[0230] *Animals*. Balb/c mice (8 weeks) were purchased from the National Laboratory Animal Center, Taiwan. All the mice were maintained in a specific pathogen-free environment. Eight-week-old Balb/c mice were immunized i.m. twice at 2-week intervals. Each vaccination contains PBS (100 μl). Sera collected from immunized mice were subjected to ELISA analysis 10 days after the last immunization. The experimental protocol was approved by Academia Sinica's Institutional Animal Care and Utilization Committee (approval no. 22-08-1901).

[0231] *LNPs*. For neutralization assay, LNPs, according to an embodiment of the present disclosure, were prepared for this experiment. Two control LNPs were also prepared to compare the performance of the present disclosure's LNPs. The first control LNP was formed using SM-102 and

DSPC ("L1+L2") without using the compound of the present disclosure. The second control LNP was a Moderna product for Spikevax ("LNP(M)"). All tested LNPs carried mRNA cargo encoding SARC-CoV-2 spike protein. For IgG titer assay, LNPs of the present disclosure were prepared to carry either a mRNA encoding wild-type SARC-CoV-2 spike protein or a mRNA encoding wild-type SARC-CoV-2 spike protein with low-sugar modification.

[0232] *Animal Immunizations.* BALB/c mice aged 6 to 8 wk old (n = 5) were immunized intramuscularly with 15 μg of LNPs in phosphate-buffered saline (PBS). Animals were immunized at week 0 and boosted with a second vaccination at week 2, and serum samples were collected from each mouse 2 weeks after the second immunization.

[0233] *Pseudovirus neutralization assay.* Pseudovirus was constructed by the RNAi Core Facility at Academia Sinica using a procedure similar to that described previously. Briefly, the pseudotyped lentivirus carrying SARS-CoV-2 spike protein was generated by transiently transfecting HEK-293T cells with pCMV-ΔR8.91, pLAS2w.Fluc.Ppuro. HEK-293T cells were seeded one day before transfection, and indicated plasmids were delivered into cells using TransITR-LT1 transfection reagent (Mirus). The culture medium was refreshed at 16 hours and harvested at 48 hours and 72 hours post-transfection. Cell debris was removed by centrifugation at 4,000 xg for 10 min, and the supernatant was passed through a 0.45-μm syringe filter (Pall Corporation). The pseudotyped lentivirus was aliquot and then stored at -80°C. To estimate the lentiviral titer by AlarmaBlue assay (Thermo Scientific), The transduction unit (TU) of SARS-CoV-2 pseudotyped lentivirus was estimated by using cell viability assay in responded to the limited dilution of lentivirus. In brief, HEK-293T cells stably expressing the human ACE2 gene were plated on a 96-well plate one day before lentivirus transduction. For the tittering pseudotyped lentivirus, different amounts of lentivirus were added into the culture medium containing polybrene (final concentration 8 μg/ml). Spin infection was carried out at 1,100 xg in a 96-well plate for 30 minutes at 37 °C. After incubating cells at 37°C for 16 hr, the culture medium containing virus and polybrene was removed and replaced with fresh complete DMEM containing 2.5 μg/ml puromycin. After treating puromycin for 48 hrs, the culture media was removed, and the cell viability was detected using 10% AlamarBlue reagents according to the manufacturer's instructions. The survival rate of uninfected cells (without puromycin treatment) was set as 100%. The virus titer (transduction units) was determined by plotting the survival cells versus the diluted viral dose. For neutralization assay, heat-inactivated sera or antibodies were serially diluted and incubated with 1,000 TU of SARS-CoV-2 pseudotyped lentivirus in DMEM for 1 h at 37°C. The mixture was then inoculated with 10,000 HEK-293T cells stably expressing the human ACE2 gene in a 96-well plate. The culture medium was replaced with fresh complete DMEM (supplemented with 10% FBS and 100 U/mL penicillin/streptomycin) at 16 h postinfection and

continuously cultured for another 48 h. The expression level of the luciferase gene was determined by using the Bright-Glo Luciferase Assay System (Promega). The relative light unit (RLU) was detected by Tecan i-control (Infinite 500). The percentage of inhibition was calculated as the ratio of RLU reduction in the presence of diluted serum to the RLU value of no serum control using the formula ($RLU^{control}$ - RLU^{Serum})/RLU control.

[0234] *Measurement of serum IgG titer*. ELISA was used to determine the IgG titer of the mouse serum. The wells of a 96-well ELISA plate (Greiner Bio-One) were coated with 100 ng SARS-CoV-2 spike protein (ACROBiosystems, wild-type, Delta, or Omicron, respectively) in 100mM sodium bicarbonate pH 8.8 at 4°C overnight. The wells were blocked with 200 µl 5% skim milk in 1X PBS at 37 °C for 1 hour and washed with 200 µl PBST (1X PBS, 0.05% Tween 20, pH 7.4) three times. Mice serum samples with 2-fold serial dilution were added into wells for incubation at 37 °C for 2 hours and washed with 200 µl PBST six times. The wells were incubated with 100 µl HRP conjugated anti-mouse secondary antibody (1:10000, in PBS) at 37 °C for 1 hour and washed with 200 µl PBST six times. 100 µl horseradish peroxidase substrate (1-Step™ Ultra TMB-ELISA Substrate Solution) (Thermo Scientific™) was added into wells, followed by 100 µl 1M H_2SO_4. After incubation for 30 minutes, absorbance (OD 450 nm) was measured using SpectraMax M5.

[0235] *Results*. **FIG. 9** shows that all tested LNPs carrying the mRNA cargo were able to deliver and express the mRNA *in vivo*, thereby invoking immune responses that resulted in neutralization inhibition. Nevertheless, the inhibitory effect of those tested LNPs differed as the dilution factor increased. Both L1+L2 LNP and LNP (M) only showed a slightly higher inhibitory effect at 1:5000 dilution compared with the negative control, but the LNP of the present disclosure maintained around 40% inhibitory effect. The data demonstrates that the LNP of the present disclosure was able to invoke immune responses at a much lower concentration than other LNPs tested in this experiment.

[0236] **FIG. 10** verifies that the LNPs of the present disclosure were capable of inducing antigen-specific IgG *in vivo*. The LNP, carrying mRNA encoding wide-type spike protein, induced IgGs that were still able to recognize the spike proteins of both the Delta variant and Omicron variant at a good level. The data shows that the targeted delivery feature of the LNP can at least partially overcome the immune escape due to the spike protein variations between variants.

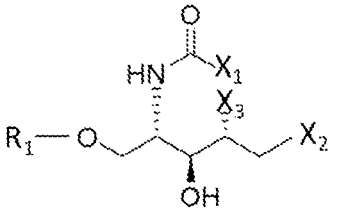
[0237] Furthermore, it was observed that LNP carrying wild-type SARS-CoV-2 spike protein-encoding mRNA ("WT LNP") and LNP carrying mRNA encoding a low-sugar modified spike protein ("low-sugar LNP") induced comparable IgG titers against wide-type viruses, the WT LNP had lower IgG

titers against the Delta and Omicron strains, suggesting an immune escape. In contrast, the low-sugar LNP maintains a high level of IgG titers against the two variant strains. The results demonstrate that removing glycan shields improves the immunogenicity of the LNP formulations.

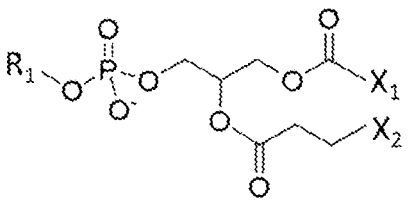
[0238] **FIG. 11** shows the results of an additional experiment. In this experiment, a Moderna LNP was prepared using Moderna's proprietary formulation. In addition, LNP made using the Moderna formulation and adding a compound of the present disclosure was also prepared to test whether the compound of the present disclosure improves the performance of the Moderna formulation. Animals were administered with the LNPs, and IgG titer assay and neutralization assay were all performed as described above in this example. The results demonstrate that the compound of the present disclosure increased the spike protein-specific IgGs. The serum obtained from mice administered with the LNP of the present disclosure's compound also exhibited better neutralization. This experiment confirmed the targeted delivery feature of the compound of the present disclosure and verified that it can be applied to commercially available LNP formulations.

[0239] **EXEMPLARY EMBODIMENTS**

[0240] Embodiment 1. A compound for forming a lipid nanoparticle, wherein the component comprises the formula:



Formula 1; or



Formula 2;

wherein R_1 comprises a substituted or non-substituted glycosyl group; 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.

[0241] Embodiment 2. The compound of embodiment 1, wherein R_1 comprises a formula of R_2-R_A-, wherein R_A is an attachment group and R_2 is the substituted or non-substituted glycosyl group, and wherein the attachment group comprises an aryl, an alkyl, an amide, an alkylamide, a substituted version thereof, a combination thereof, or a covalent bond.

[0242] Embodiment 3. The compound of embodiment 2, wherein R_A comprises the aryl having 0 to 3 substituents, wherein the substituent is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide.

[0243] Embodiment 4. The compound of embodiment 3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.

[0244] Embodiment 5. The copolymer of embodiment 3 or embodiment 4, wherein the PEG moiety is linear.

[0245] Embodiment 6. The compound of any one of embodiments 1 to 5, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.

[0246] Embodiment 7. The compound of any one of embodiments 1 to 6, wherein the glycosyl group comprises a terminal mannoside, a terminal fucoside, or both.

[0247] Embodiment 8. The compound of any one of embodiments 1 to 7, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.

[0248] Embodiment 9. The compound of embodiment 8, wherein the tri-mannoside is a linear or branched tri-mannoside.

[0249] Embodiment 10. The compound of embodiment 9, wherein the branched tri-mannoside is a α-1,3-α-1,6-trimannoside.

[0250] Embodiment 11. The compound of any one of embodiments 1 to 10, wherein R_1 is a substituted glycosyl group.

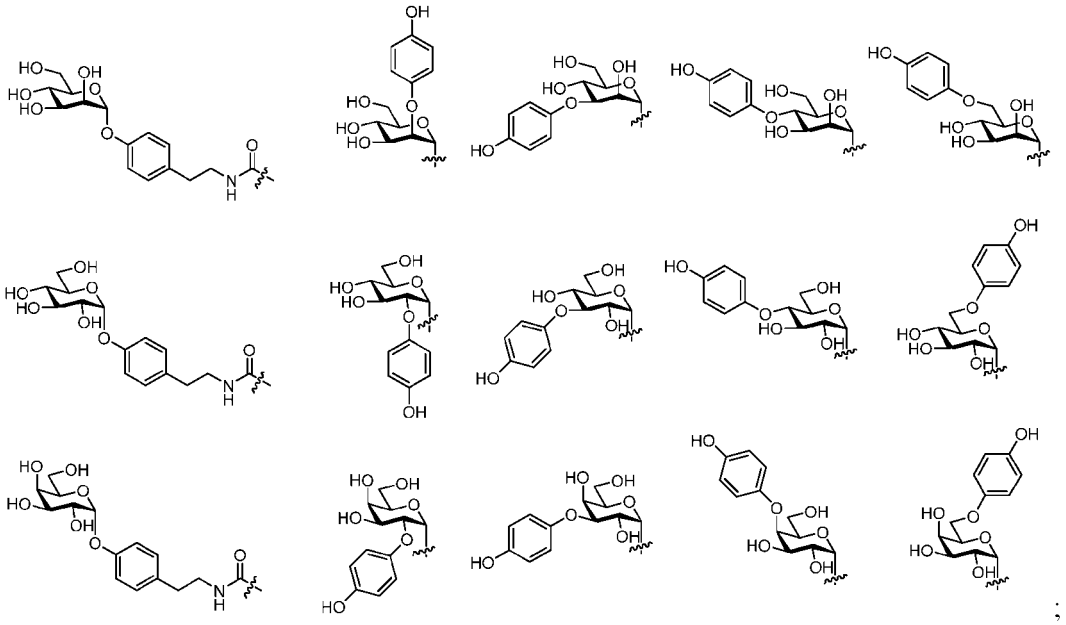
[0251] Embodiment 12. The compound of embodiment 11, wherein the glycosyl group comprises 1 to 6 substituents, wherein the substituent is C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, C_{1-6} alkoxy, amine, nitro, C_{1-6} alkyl amine, amide, azido, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

[0252] Embodiment 13. The compound of embodiment 12, wherein the substituent of the glycosyl group is selected from the group consisting of aryl, 5-membered cycloalkyl, 6-membered cycloalkyl, 5-membered heterocycloalkyl, and 6-membered heterocycloalkyl, and a substituted version thereof, which comprises 1 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halide, C_{1-6} alkoxy, amine, nitro, C_{1-6} alkyl amine, amide, azido, carboxyl, hydroxyl, aryl, cycloalkyl, heterocycloalkyl, or a substituted version thereof, or a combination thereof.

[0253] Embodiment 14. The compound of embodiment 12 or embodiment 13, wherein the substituent of the glycosyl group is a substituted or non-substituted aryl, optionally the substituent of the glycosyl group is a phenyl substituted with OH, CH_3, NH_2, CF_3, OCH_3, F, Br, Cl, NO_2, N_3, or a combination thereof.

[0254] Embodiment 15. The compound of embodiment 12 or embodiment 13, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.

[0255] Embodiment 16. The compound of any one of embodiments 1 to 15, wherein R_1 is selected from the group consisting of:



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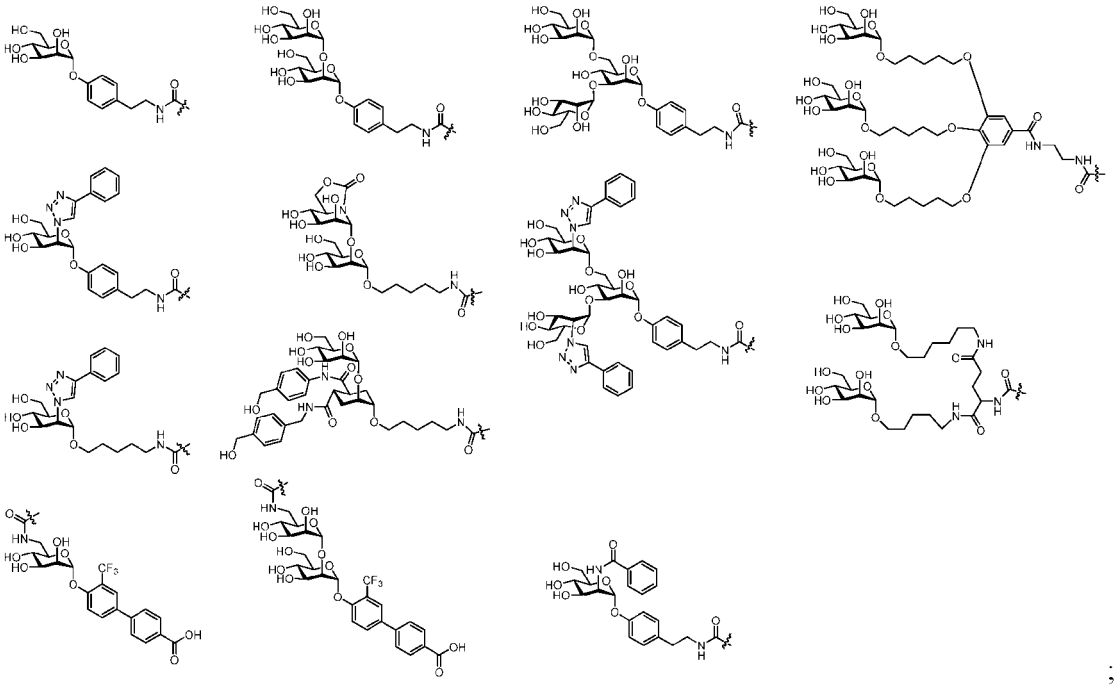

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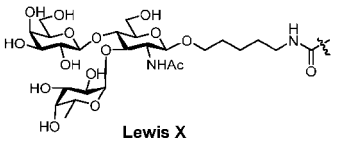
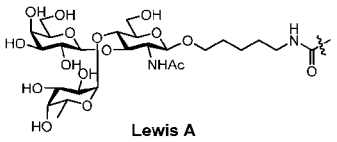
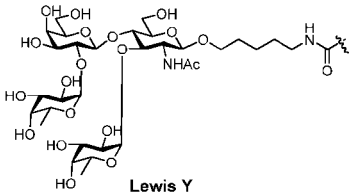


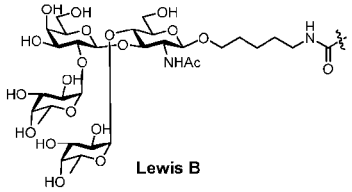
Lewis X



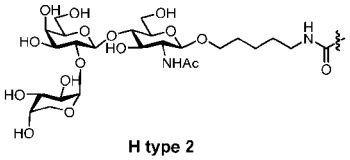
Lewis A



Lewis Y



Lewis B



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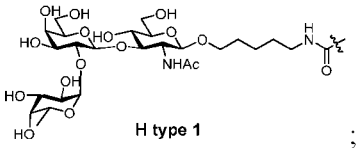

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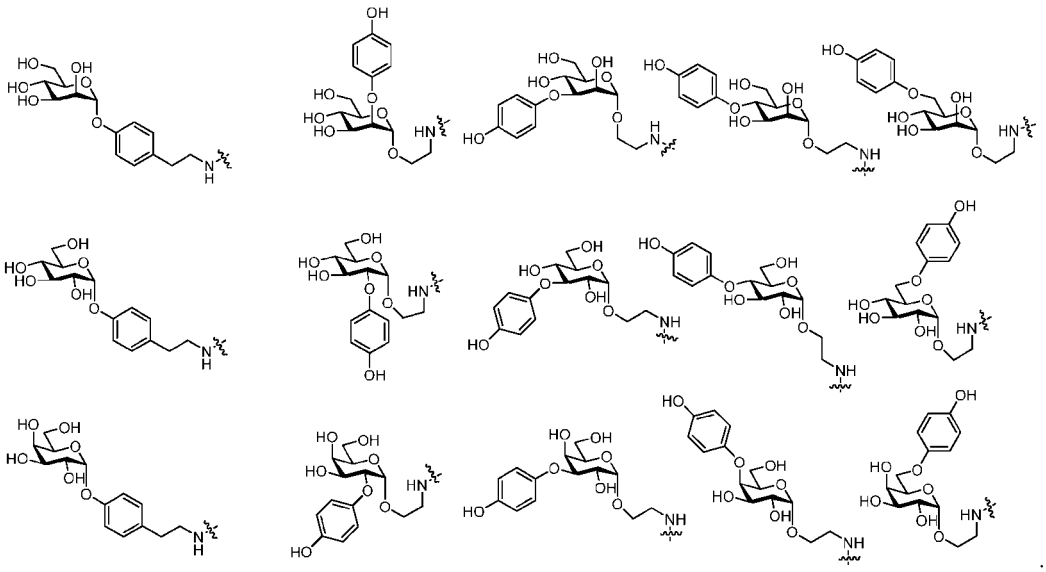
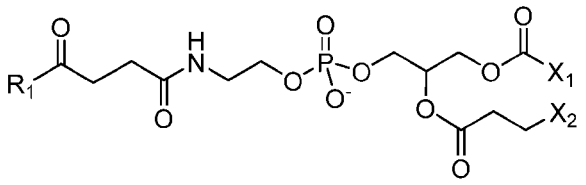


[0256] Embodiment 17. The compound of any one of embodiments 1 to 16, wherein the compound is of Formula 1.

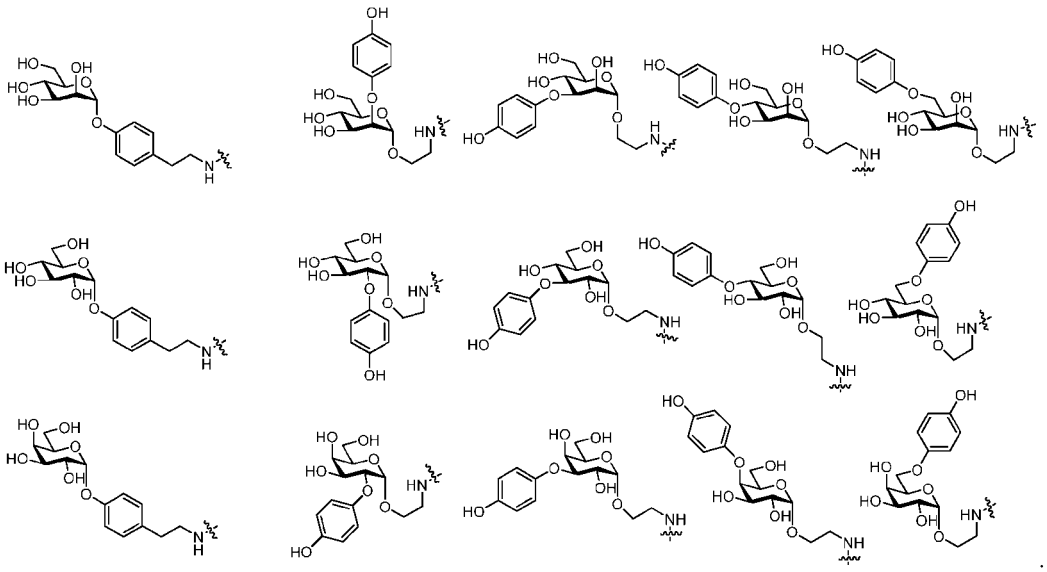
[0257] Embodiment 18. The compound of any one of embodiments 1 to 16, wherein the compound is of Formula 2.

[0258] Embodiment 19. The compound of embodiment 18, wherein the compound is of Formula 3:



Formula 3; and

wherein R_1 is selected from the group consisting of:



[0259] Embodiment 20. The compound of any one of embodiments 1 to 19, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising 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.

[0260] Embodiment 21. The compound of any one of embodiments 1 to 20, 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.

[0261] Embodiment 22. The compound of embodiment 21, wherein X_1 and X_2 are each independently hydrogen, $C_{8\text{-}30}$ alkyl, $C_{8\text{-}30}$ alkenyl, $C_{8\text{-}30}$ alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)nX_4$, n is 8 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.

[0262] Embodiment 23. The compound of any one of embodiments 1 to 22, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen.

[0263] Embodiment 24. The compound of any one of embodiments 1 to 23, 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.

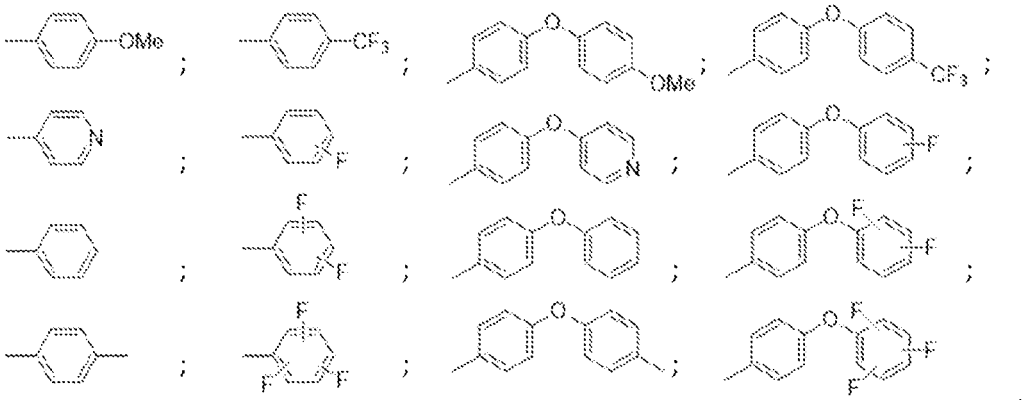
[0264] Embodiment 25. The compound of embodiment 24, wherein the substituent is CH_3, CF_3, F, or OCH_3.

[0265] Embodiment 26. The compound of embodiment 24 or embodiment 25, wherein X_4 comprises 1 to 3 substituents.

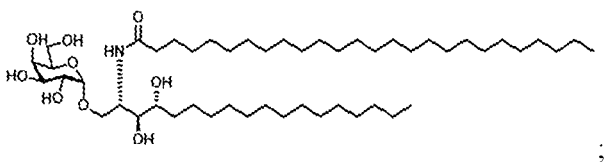
[0266] Embodiment 27. The compound of any one of embodiments 24 to 26, wherein X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

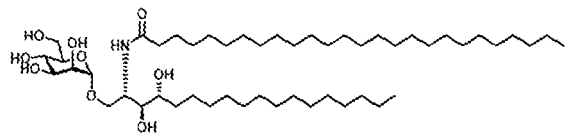
[0267] Embodiment 28. The compound of any one of embodiments 1 to 27, wherein one of X_1 and X_2 is C_{15-30} alkyl, and the other one is $-(CH_2)nX_4$.

[0268] Embodiment 29. The compound of any one of embodiments 1 to 28, wherein X_4 is selected from the group consisting of:



[0269] Embodiment 30. The compound of any one of embodiments 1 to 29, selected from the group consisting of:





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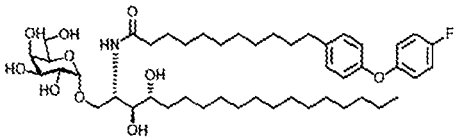

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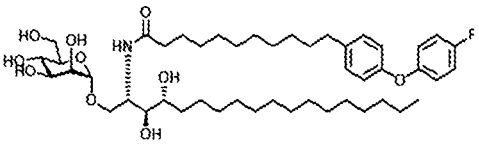

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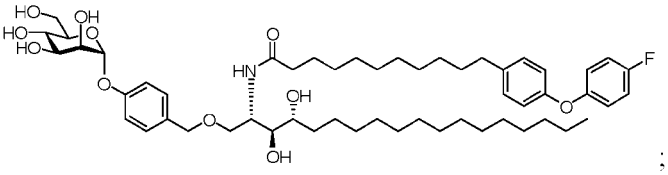

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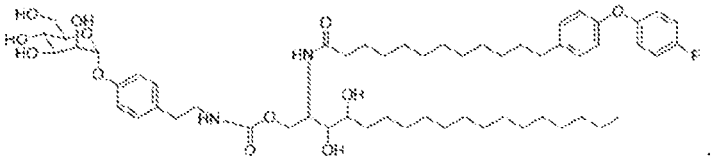

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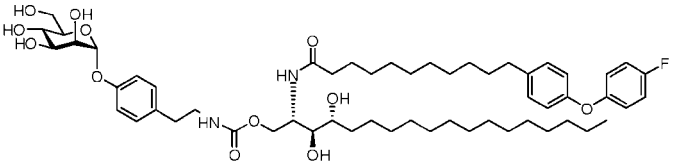

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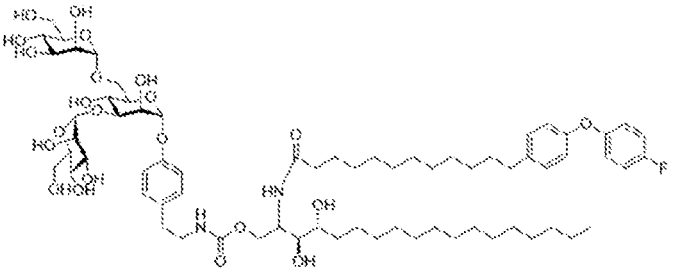

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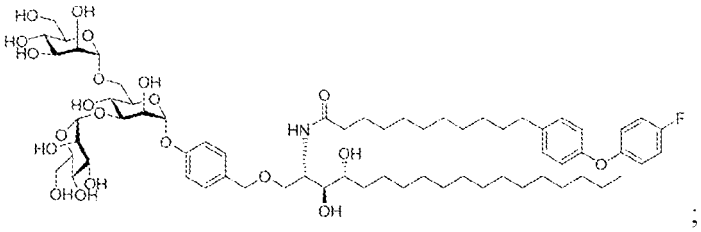
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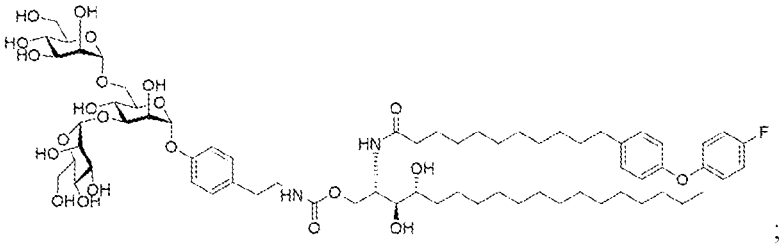
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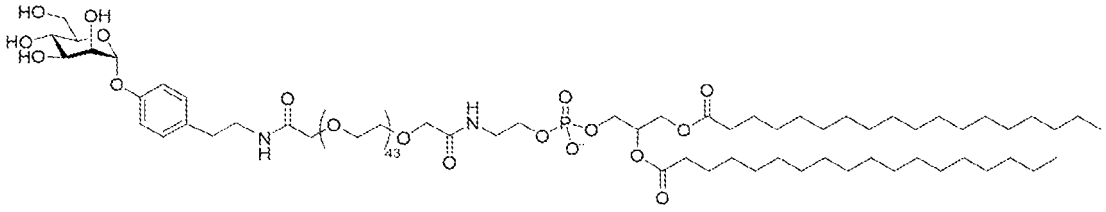
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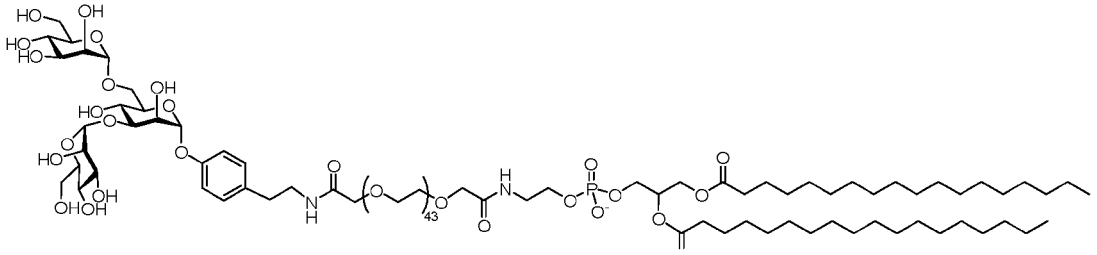
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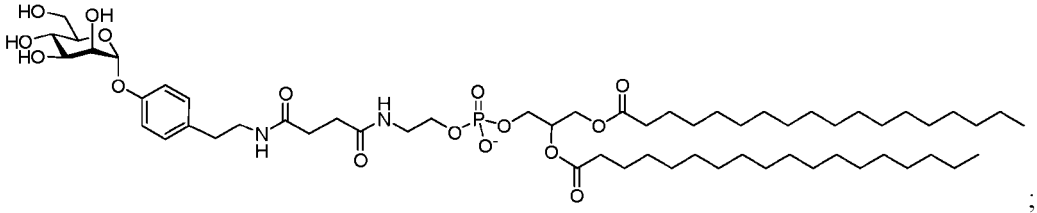
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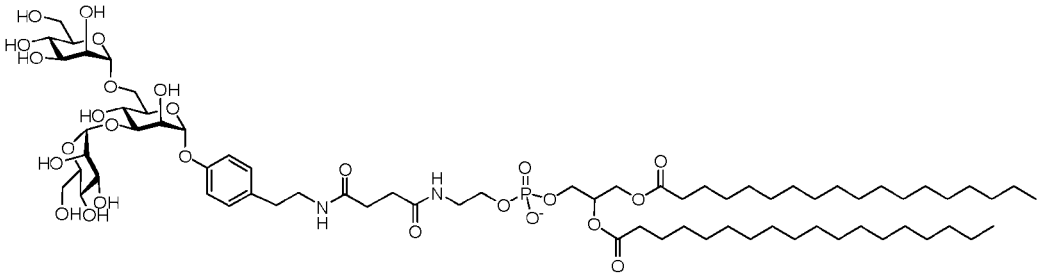
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[0270] Embodiment 31. The component of any one of embodiments 1 to 30, wherein the component is not glycolipid C34 or α-galactosylceramide.

[0271] Embodiment 32. A formulation for forming a lipid nanoparticle, comprising the compound of any one of embodiments 1 to 31, wherein the compound comprises 1 to 10 mol% of the composition.

[0272] Embodiment 33. The formulation of embodiment 32, further comprises an ionizable lipid, a helper lipid, or a mixture thereof, wherein the ionizable lipid comprises 30 to 60 mol% of the composition, the helper lipid comprises 5 to 60 mol% of the composition, and the rest percent is a carrier or a solvent.

[0273] Embodiment 34. The formulation of embodiment 33, 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.

[0274] Embodiment 35. The formulation of embodiment 33 or embodiment 34, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof, wherein the phosphatidylcholine comprises 5 to 10 mol% of the composition, the cholesterol or a derivative thereof comprises 30 to 40 mol% of the composition, and the polyethylene glycol-lipid (PEG-lipid) comprises 1 to 10 mol% of the composition.

[0275] Embodiment 36. The formulation of embodiment 35, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0276] Embodiment 37. The formulation of embodiment 35 or embodiment 36, wherein the cholesterol or a derivative thereof is a cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

[0277] Embodiment 38. The formulation of any one of embodiments 35 to 37, wherein the PEG-lipid is DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-

amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof.

[0278] Embodiment 39. The formulation of any one of embodiments 33 to 38, further comprising a payload.

[0279] Embodiment 40. The formulation of embodiment 39, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0280] Embodiment 41. The formulation of embodiment 40, wherein the nucleic acid is RNA or DNA.

[0281] Embodiment 42. The formulation of embodiment 41, wherein the payload encodes a polypeptide.

[0282] Embodiment 43. The formulation of any one of embodiments 39 to 42, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0283] Embodiment 44. The formulation of any one of embodiments 39 to 43, wherein the payload is a first payload, and the composition further encapsulates a second payload, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0284] Embodiment 45. The formulation of embodiment 44, wherein the first payload and the second payload are different.

[0285] Embodiment 46. The formulation of any one of embodiments 32 to 45, further comprising a pharmaceutically acceptable excipient, adjuvant, or a combination thereof.

[0286] Embodiment 47. A lipid nanoparticle, comprising a membrane defining an inner space, wherein the membrane is formed with a plurality of lipid components comprising the compound of any one of embodiments 1 to 31.

[0287] Embodiment 48. The lipid nanoparticle of embodiment 47, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof.

[0288] Embodiment 49. The lipid nanoparticle of embodiment 47 or embodiment 48, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.

[0289] Embodiment 50. The lipid nanoparticle of embodiment 49, 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.

[0290] Embodiment 51. The lipid nanoparticle of embodiment 49 or embodiment 50, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

[0291] Embodiment 52. The lipid nanoparticle of embodiment 51, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0292] Embodiment 53. The lipid nanoparticle of embodiment 51 or embodiment 52, wherein the cholesterol or a derivative thereof is a cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

[0293] Embodiment 54. The lipid nanoparticle of any one of embodiments 51 to 53, wherein the PEG-lipid is DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof.

[0294] Embodiment 55. The lipid nanoparticle of any one of embodiments 47 to 54, wherein the membrane encapsulates a payload.

[0295] Embodiment 56. The lipid nanoparticle of embodiment 55, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0296] Embodiment 57. The lipid nanoparticle of embodiment 56, wherein the nucleic acid is RNA or DNA.

[0297] Embodiment 58. The lipid nanoparticle of embodiment 57, wherein the payload encodes a polypeptide.

[0298] Embodiment 59. The lipid nanoparticle of any one of embodiments 55 to 58, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0299] Embodiment 60. The lipid nanoparticle of any one of embodiments 55 to 59, wherein the payload is a first payload, and the membrane further encapsulates a second payload.

[0300] Embodiment 61. The lipid nanoparticle of embodiment 60, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0301] Embodiment 62. The lipid nanoparticle of embodiment 60 or embodiment 61, wherein the first payload and the second payload are different.

[0302] Embodiment 63. The lipid nanoparticle of any one of embodiments 47 to 62, being made from the composition of any one of embodiments 29 to 43.

[0303] Embodiment 64. The lipid nanoparticle of any one of embodiments 47 to 63, wherein the membrane is a bi-layer structure.

[0304] Embodiment 65. The lipid nanoparticle of any one of embodiments 47 to 64, having a diameter of 0.01 to 5 microns.

[0305] Embodiment 66. The lipid nanoparticle of any one of embodiments 47 to 65, wherein the plurality of the lipid components does not comprise glycolipid C34 or α-galactosylceramide (α-GalCer).

[0306] Embodiment 67. A formulation, comprising the lipid nanoparticle of any one of embodiments 47 to 66.

[0307] Embodiment 68. The formulation of embodiment 67, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.

[0308] Embodiment 69. The formulation of embodiment 67 or embodiment 68, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.

[0309] Embodiment 70. The formulation of embodiment 69, wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.

[0310] Embodiment 71. The formulation of any one of embodiments 67 to 70, further comprising an excipient, an adjuvant, or a combination thereof.

[0311] Embodiment 72. The formulation of embodiment 71, 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.

[0312] Embodiment 73. The formulation of embodiment 71 or embodiment 72, 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.

[0313] Embodiment 74. A kit for preparing a lipid nanoparticle, comprising: a first reagent, comprising the compound of any one of embodiments 1 to 31; and a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.

[0314] Embodiment 75. The kit of embodiment 74, wherein the second reagent comprises the ionizable lipid.

[0315] Embodiment 76. The kit of embodiment 75, wherein the kit further comprises a third reagent comprising a helper lipid.

[0316] Embodiment 77. The kit of any one of embodiments 74 to 76, wherein 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.

[0317] Embodiment 78. The kit of any one of embodiments 74 to 77, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

[0318] Embodiment 79. The kit of embodiment 78, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0319] Embodiment 80. The kit of embodiment 78 or embodiment 79, wherein the cholesterol or a derivative thereof is a cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

[0320] Embodiment 81. The kit of any one of embodiments 78 to 80, wherein the PEG-lipid is DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof.

[0321] Embodiment 82. The kit of any one of embodiments 74 to 81, further comprising a fourth reagent, comprising a payload, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0322] Embodiment 83. The kit of embodiment 82, wherein the nucleic acid is RNA or DNA.

[0323] Embodiment 84. The kit of embodiment 83, wherein the payload encodes a polypeptide.

[0324] Embodiment 85. The kit of any one of embodiments 82 to 84, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0325] Embodiment 86. A method of targeted payload delivery in a subject, comprising administering to the subject an effective amount of the lipid nanoparticle of any one of embodiments 47 to 54, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated by the lipid nanoparticle.

[0326] Embodiment 87. The method of embodiment 86, wherein the nucleic acid is RNA or DNA.

[0327] Embodiment 88. The method of embodiment 87, wherein the payload encodes a polypeptide.

[0328] Embodiment 89. The method of any one of embodiments 86 to 88, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0329] Embodiment 90. The method of any one of embodiments 86 to 89, wherein the payload is a first payload, and the membrane further encapsulates a second payload, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0330] Embodiment 91. The method of embodiment 90, wherein the first payload and the second payload are different.

[0331] Embodiment 92. The method of any one of embodiments 86 to 91, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.

[0332] Embodiment 93. The method of embodiment 92, 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.

[0333] Embodiment 94. The method of embodiment 92 or embodiment 93, 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.

[0334] Embodiment 95. A method of preventing or treating a disease in a subject, comprising administering to the subject an effective amount of the lipid nanoparticle of any one of embodiments 47 to 54, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated within the lipid nanoparticle; and wherein the payload is a therapeutic agent or derives a therapeutic agent.

[0335] Embodiment 96. The method of embodiment 95, wherein the nucleic acid is RNA or DNA.

[0336] Embodiment 97. The method of embodiment 96, wherein the payload encodes a polypeptide.

[0337] Embodiment 98. The method of any one of embodiments 95 to 97, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0338] Embodiment 99. The method of any one of embodiments 95 to 98, wherein the payload is a first payload, and the membrane further encapsulates a second payload, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0339] Embodiment 100. The method of embodiment 99, wherein the first payload and the second payload are different.

[0340] Embodiment 101. The method of any one of embodiments 95 to 100, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.

[0341] Embodiment 102. The method of embodiment 101, 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.

[0342] Embodiment 103. The method of embodiment 101 or embodiment 102, 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.

[0343] Embodiment 104. The method of any one of embodiments 95 to 103, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.

[0344] Embodiment 105. The method of embodiment 104, wherein the booster doses are administered about one month, about two months, about three months, about four months, about five months, or about six months or more following the initial dose.

[0345] Embodiment 106. The method of any one of embodiments 95 to 105, wherein the effective amount ranges from about 5 μg to 1000 μg.

[0346] Embodiment 107. A method of boosting an adaptive immune response, comprising administering to the subject an effective amount of the lipid nanoparticle of any one of embodiments 47 to 54, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the payload is encapsulated within the lipid nanoparticle; and wherein the payload is immunogenic or derives an immunogenic biomolecule.

[0347] Embodiment 108. The method of embodiment 107, wherein the nucleic acid is RNA or DNA.

[0348] Embodiment 109. The method of embodiment 108, wherein the payload encodes a polypeptide.

[0349] Embodiment 110. The method of any one of embodiments 107 to 109, wherein the payload is a first payload, and the membrane further encapsulates a second payload, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0350] Embodiment 111. The method of embodiment 110, wherein the first payload and the second payload are different.

[0351] Embodiment 112. The method of any one of embodiments 107 to 111, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.

[0352] Embodiment 113. The method of embodiment 112, 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.

[0353] Embodiment 114. The method of embodiment 112 or embodiment 113, 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.

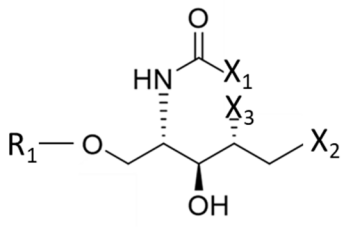
[0354] Embodiment 115. The method of any one of embodiments 107 to 114, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.

[0355] Embodiment 116. The method of embodiment 115, wherein the booster doses are administered about one month, about two months, about three months, about four months, about five months, or about six months or more following the initial dose.

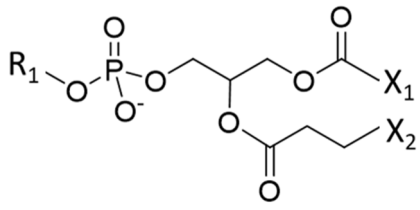
[0356] Embodiment 117. The method of any one of embodiments 107 to 116, wherein the effective amount ranges from about 5 μg to 1000 μg.

[0357] Particular features of the present disclosure are set out in the following numbered paragraphs:

1. A compound for forming a lipid nanoparticle, wherein the component comprises the formula:



Formula 1; or



Formula 2;

wherein R_1 comprises a substituted or non-substituted glycosyl group;

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.

2. The compound of paragraph 1, wherein R_1 comprises a formula of R_2-R_A-, wherein R_2 is the substituted or non-substituted glycosyl group, and R_A is an attachment group, and wherein the attachment group comprises an aryl, an alkyl, an amide, an alkylamide, a substituted version thereof, a combination thereof, or a covalent bond.

3. The compound of paragraph 2, wherein R_A comprises an aryl having 1 to 3 substituents, wherein the substituent is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide.

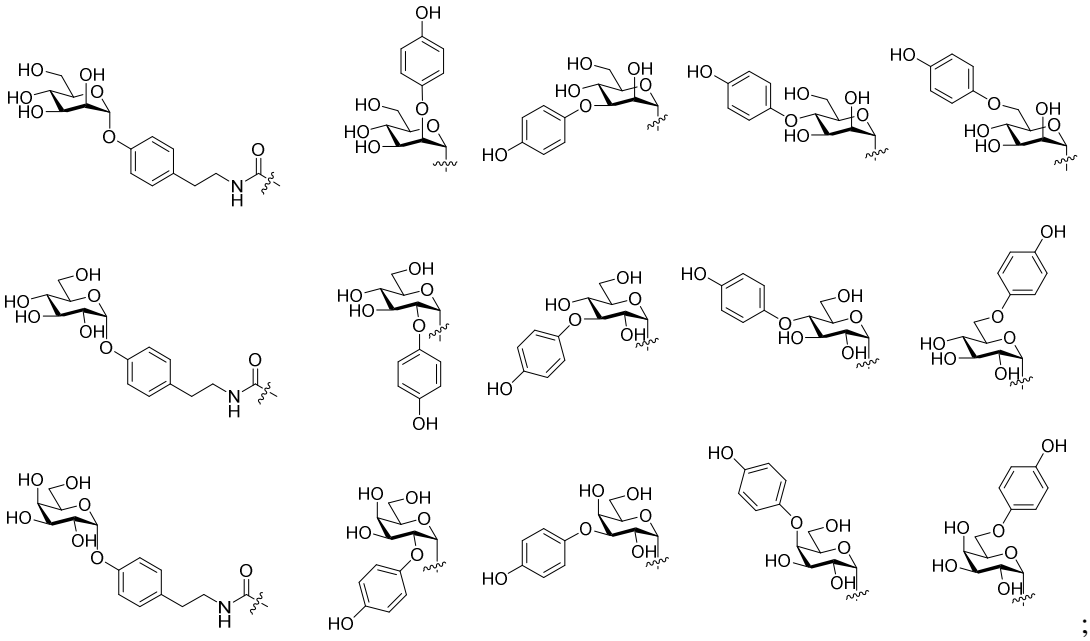
4. The compound of paragraph 3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.

5. The compound of paragraph 1, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.

6. The compound of paragraph 1, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.

7. The compound of paragraph 1, wherein R_1 is 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.

8. The compound of paragraph 1, wherein R_1 is selected from the group consisting of: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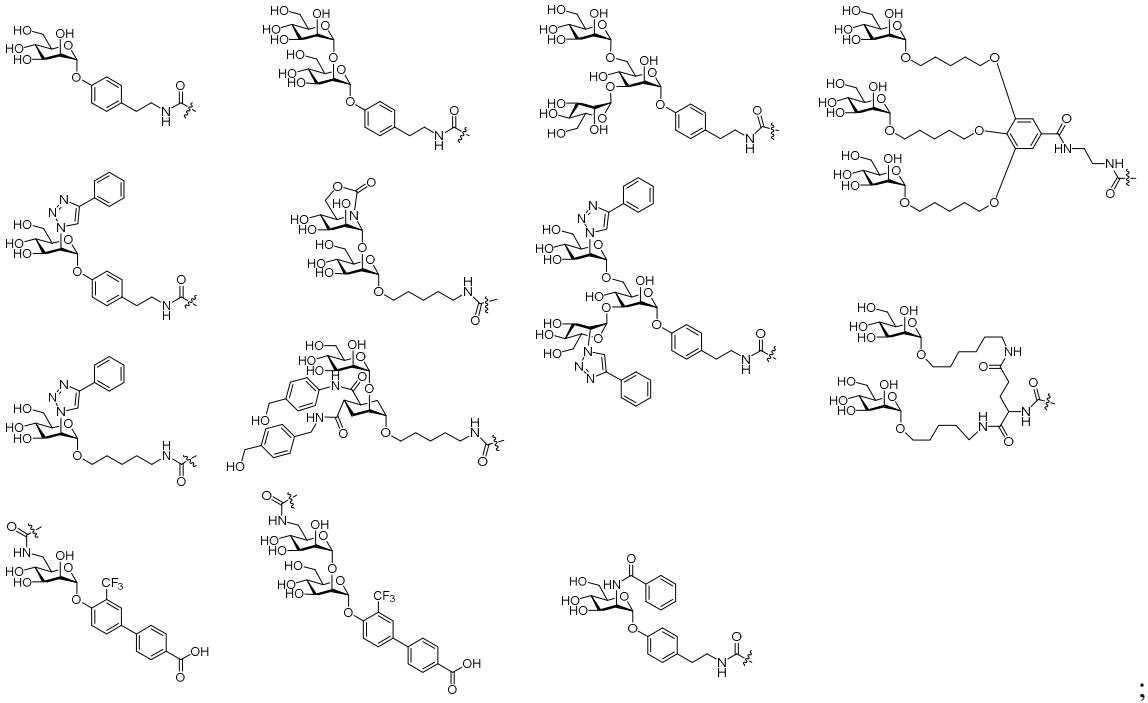

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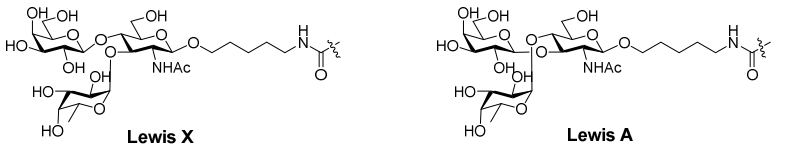
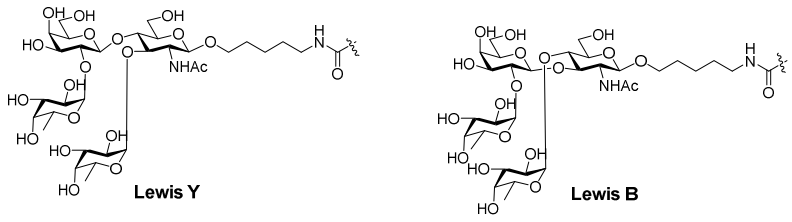
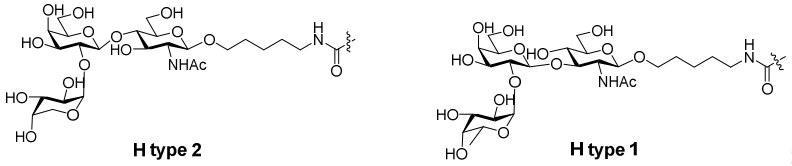

Lewis X
Lewis A


Lewis Y
Lewis B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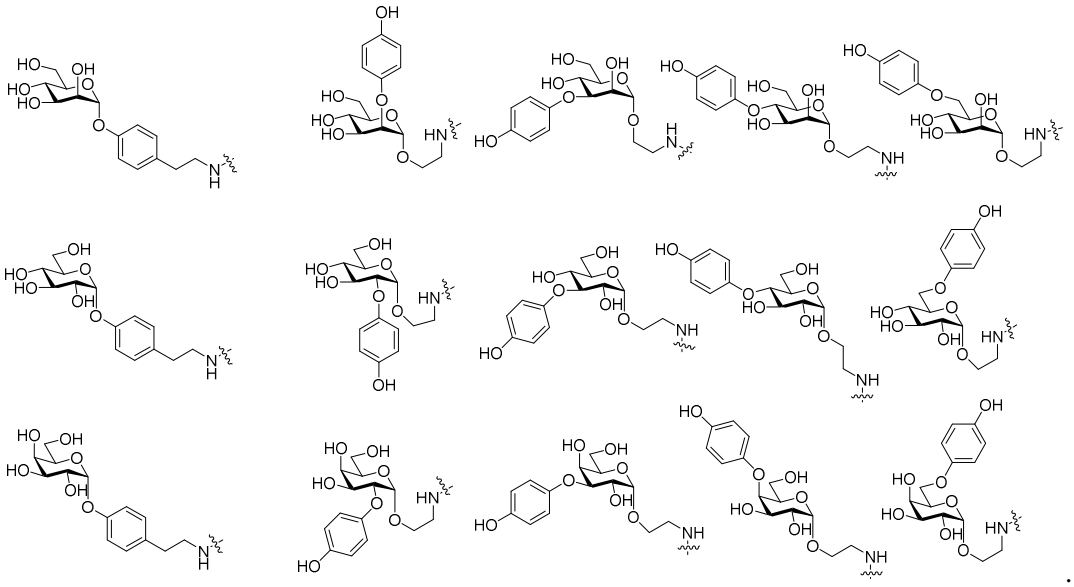
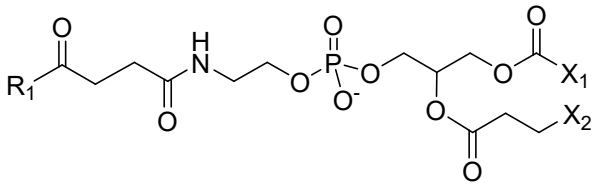


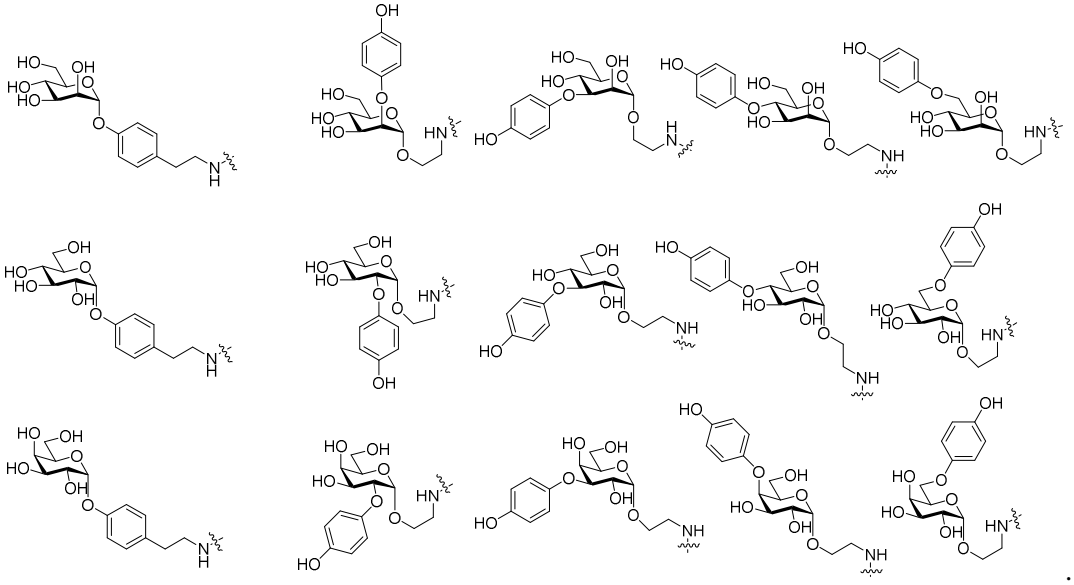
9. The compound of paragraph 1, wherein the compound is of Formula 1.

10. The compound of paragraph 1, wherein the compound is of Formula 3:



Formula 3; and

wherein R_1 is selected from the group consisting of:



11. The compound of paragraph 1, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising 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.

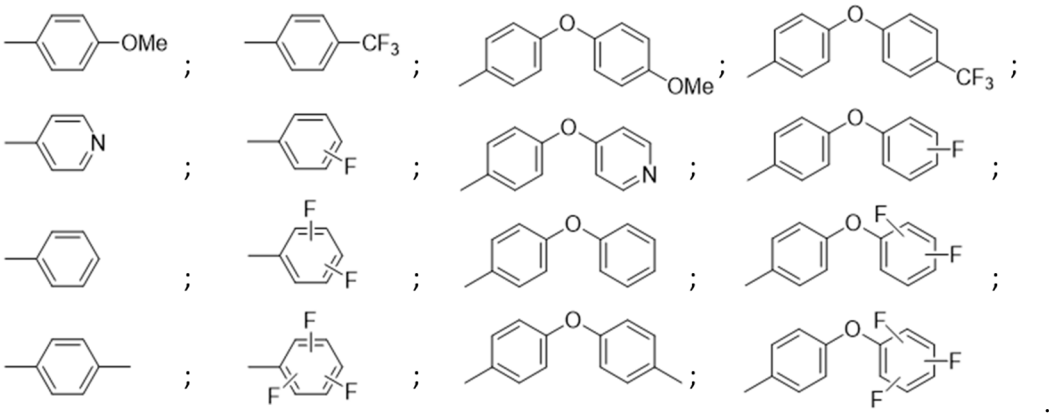
12. The compound of paragraph 1, wherein X_1 and X_2 are each independently hydrogen, C_{4-30} alkyl, C_{4-30} alkenyl, C_{4-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or

$-(CH_2)nX_4$, 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.

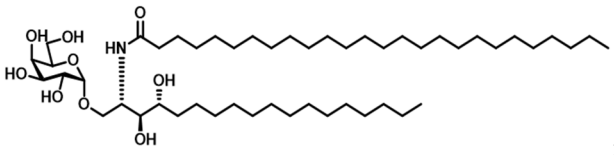
13. The compound of paragraph 1, wherein X_4 is an aryl, aryloxy, heterocyclic group, cycloalkyl, heterocycloalkyl, or a combination thereof, and wherein X_4 comprises 0 to 6 substituents, selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

14. The compound of paragraph 13, 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.

15. The compound of paragraph 1, wherein X_4 is selected from the group consisting of:



16. The compound of paragraph 1, selected from the group consisting of:



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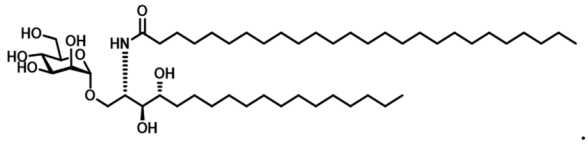

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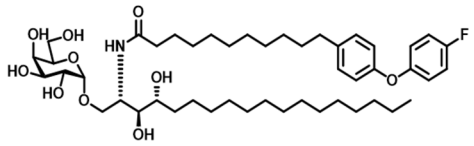

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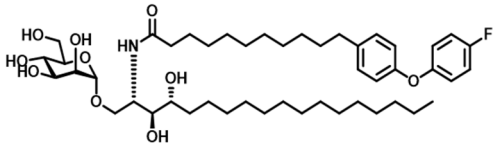

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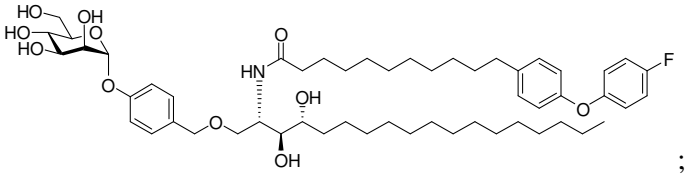

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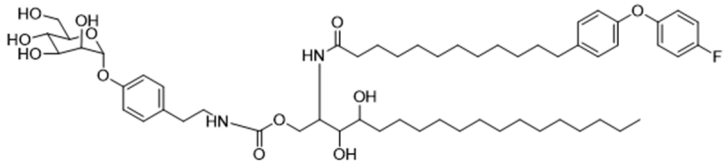

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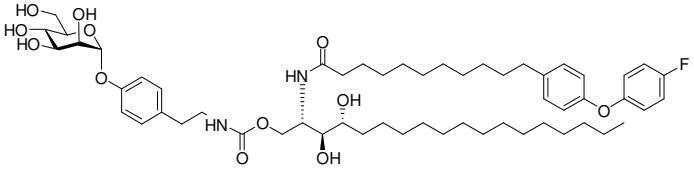

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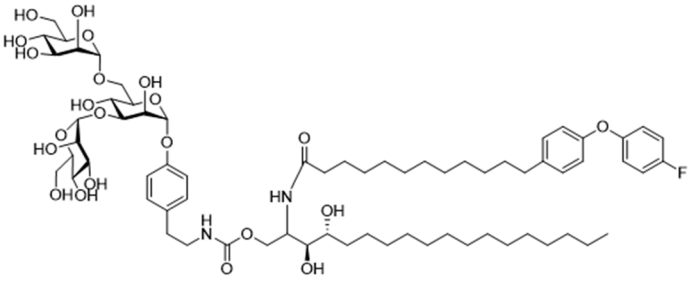

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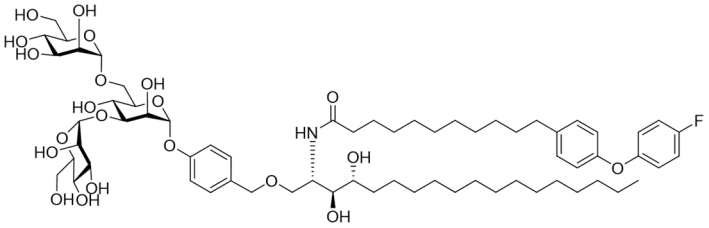

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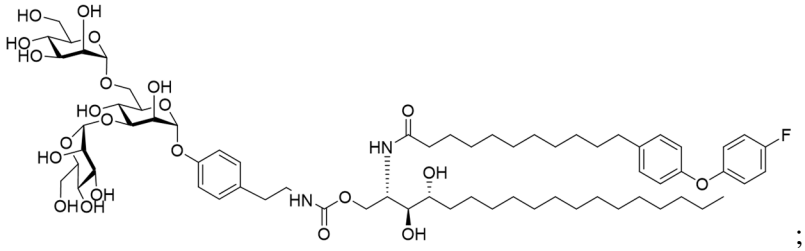
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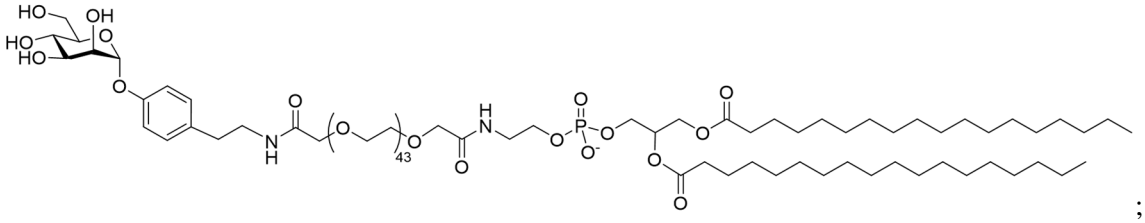
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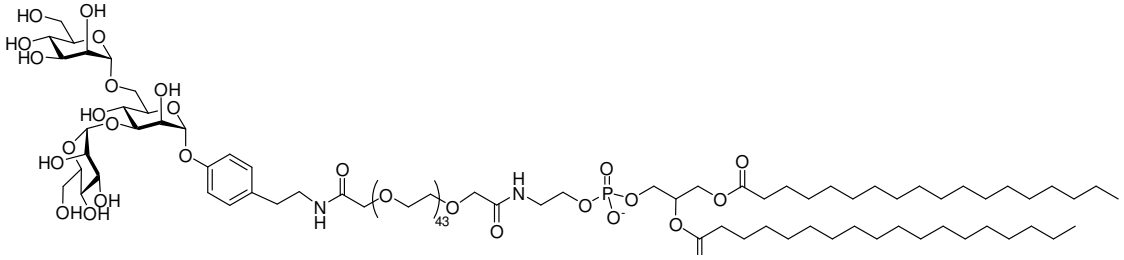
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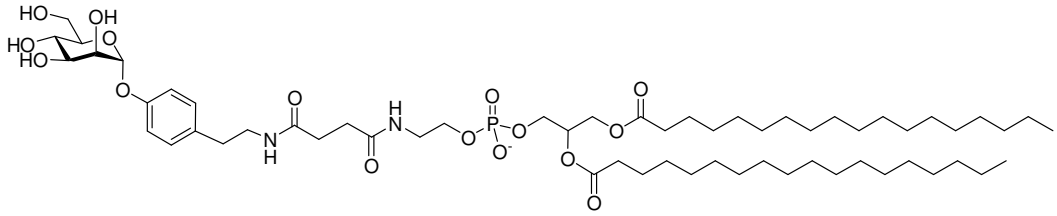
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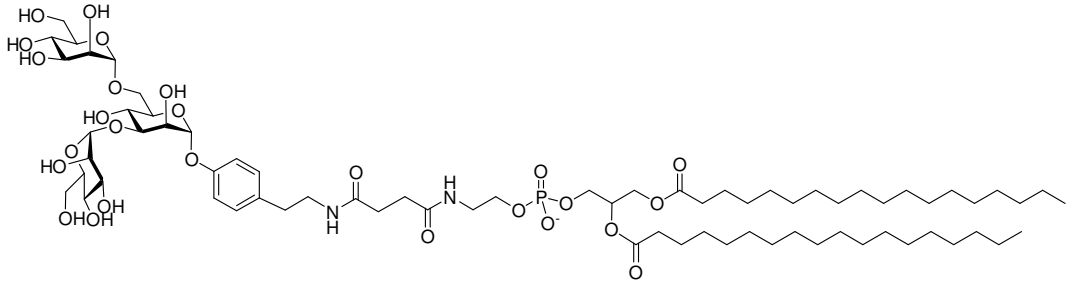

.

17. A lipid nanoparticle, comprising a membrane defining an inner space, wherein the membrane is formed with a plurality of lipid components comprising the compound of any one of paragraphs 1 to 16.

18. The lipid nanoparticle of paragraph 17, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

19. The lipid nanoparticle of paragraph 17, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.

20. The lipid nanoparticle of paragraph 19, 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.

21. The lipid nanoparticle of paragraph 17, wherein the membrane encapsulates a payload, which is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

22. The lipid nanoparticle of paragraph 21, wherein the nucleic acid is RNA or DNA encoding a polypeptide.

23. The lipid nanoparticle of paragraph 21, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

24. The lipid nanoparticle of paragraph 21, wherein the payload is a first payload, and the membrane further encapsulates a second payload.

25. A formulation, comprising the lipid nanoparticle of paragraph 17.

26. The formulation of paragraph 25, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.

27. The formulation of paragraph 25, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.

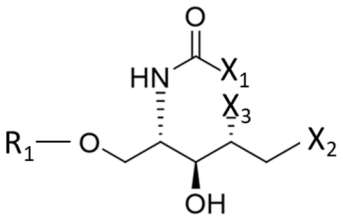
28. The formulation of paragraph 25, further comprising an excipient, an adjuvant, or a combination thereof.

29. The formulation of paragraph 28, 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.

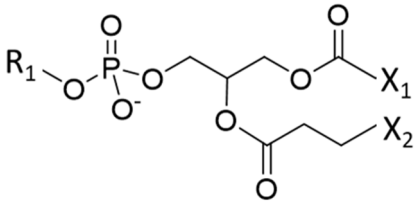
30. The formulation of paragraph 28, wherein the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, α-galactosylceramide, Aluminum salt, Squalene, MF59, or QS-21. Other examples of adjuvants in some vaccines that can be used in the composition of the present disclosure are aluminum hydroxide, 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.

The claims defining the invention are as follows:

1. A lipid nanoparticle, comprising a membrane defining an inner space, wherein the membrane is formed with a plurality of lipid components, wherein the plurality of lipid components comprises a compound comprising Formula 1 or Formula 2:



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.

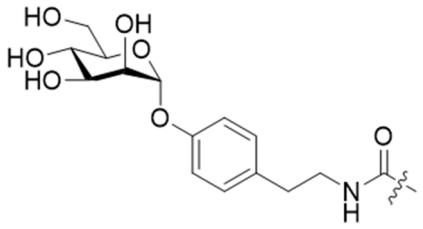
2. The lipid nanoparticle of claim 1, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.

3. The lipid nanoparticle of claim 1 or claim 2, wherein the substituted or non-substituted glycosyl group comprises mannoside, fucoside, or a combination thereof.

4. The lipid nanoparticle of any one of claims 1 to 3, wherein the substituted or non-substituted glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.

5. The lipid nanoparticle of any one of claims 1 to 4, wherein R_2 is a substituted glycosyl group, comprising 1 to 6 substituents, wherein each of the 1 to 6 substituents 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.

6. The lipid nanoparticle of any one of claims 1 to 5, wherein R_1 is selected from the group consisting of:



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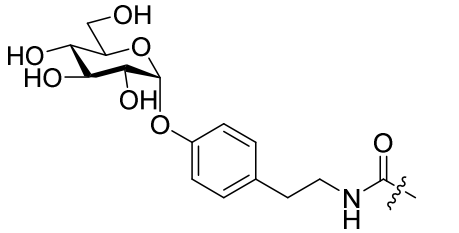

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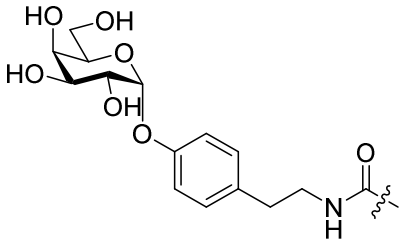

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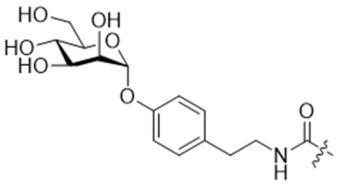
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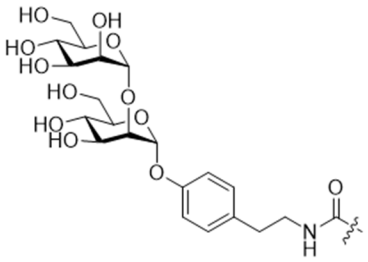
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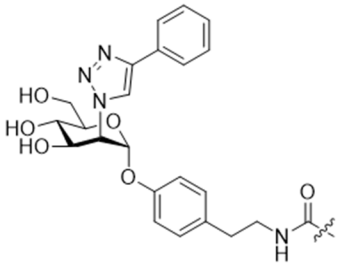
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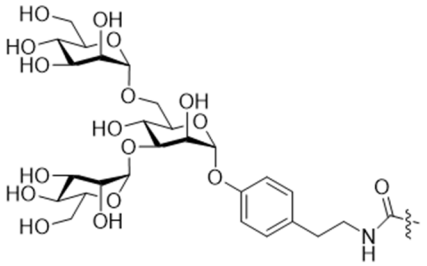
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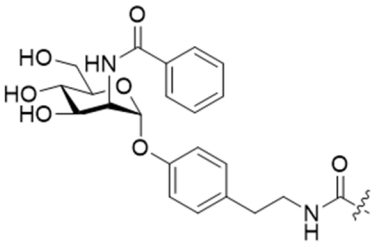
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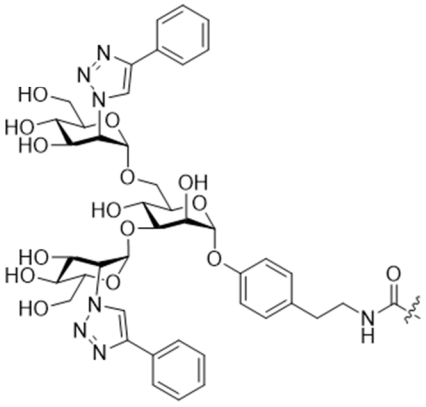
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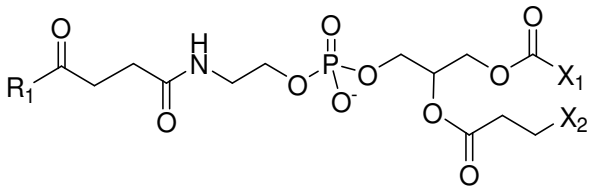
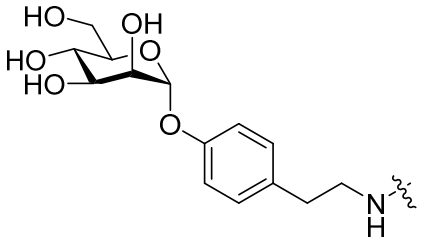
7. The lipid nanoparticle of any one of claims 1 to 6, wherein the compound is of Formula 3:



Formula 3; and

wherein R_1 is selected from the group consisting of:



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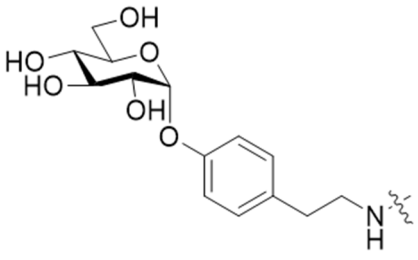
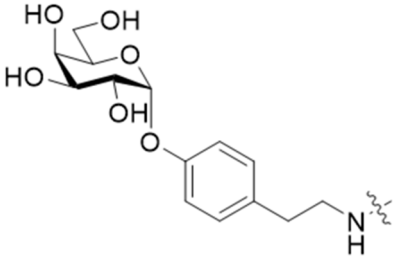


; and



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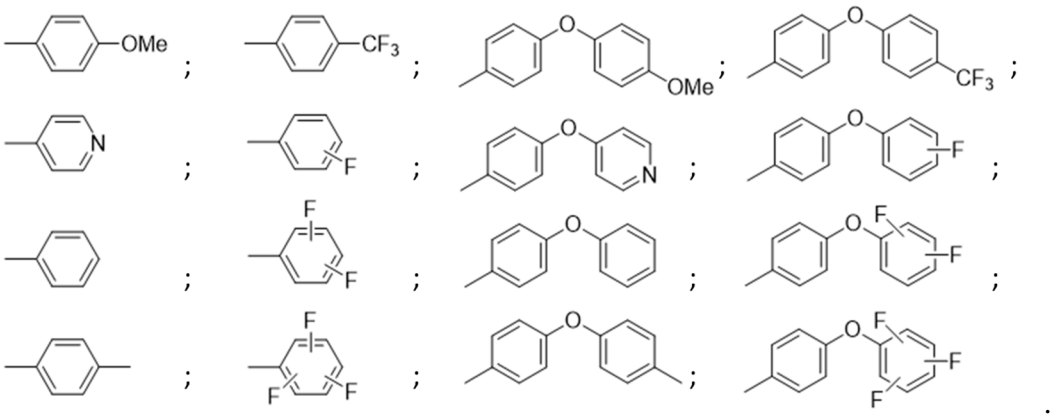
8. The lipid nanoparticle of any one of claims 1 to 7, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons; and/or

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.

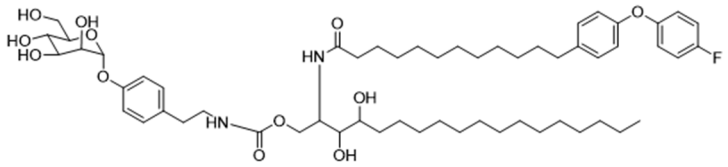
9. The lipid nanoparticle of any one of claims 1 to 8, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen, or when one of X_1 and X_2 is C_{15-30} alkyl, the other one is $–(CH_2)nX_4$.

10. The lipid nanoparticle of any one of claims 1 to 9, 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.

11. The lipid nanoparticle of any one of claims 1 to 10, wherein X_4 is selected from the group consisting of:



12. The lipid nanoparticle of any one of claims 1 to 11, wherein the compound is selected from the group consisting of:



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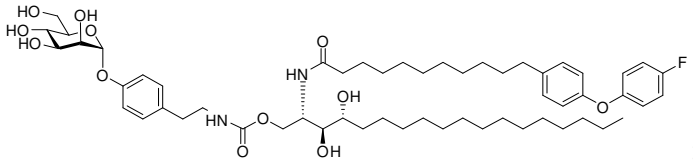

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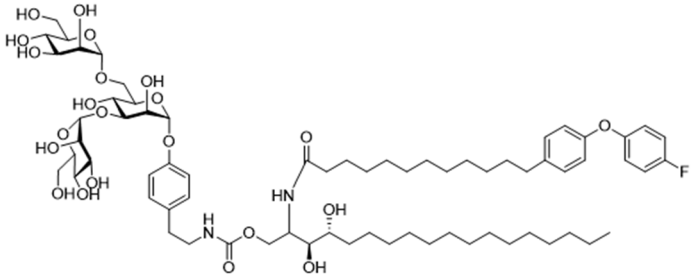

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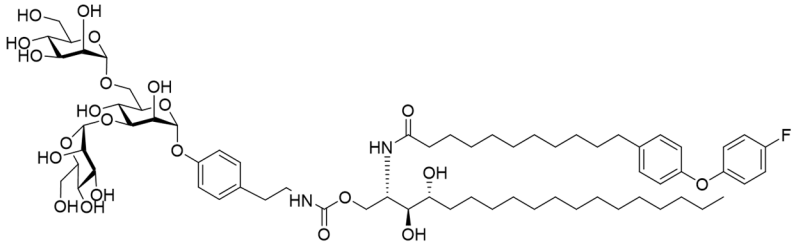

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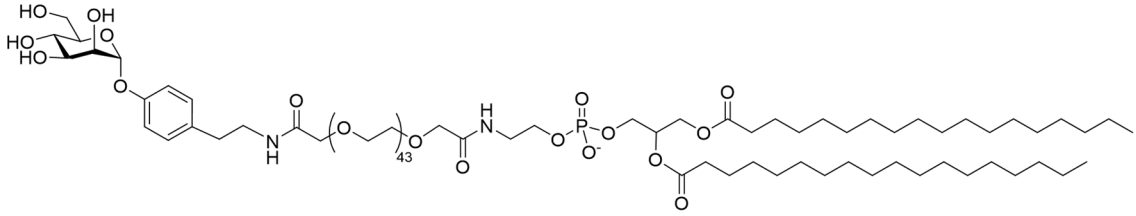

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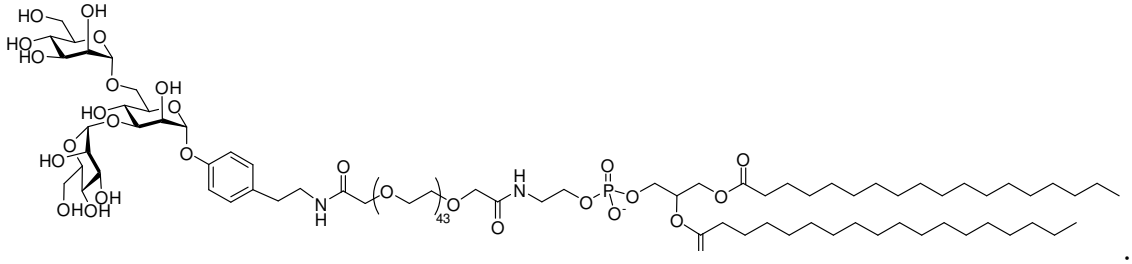

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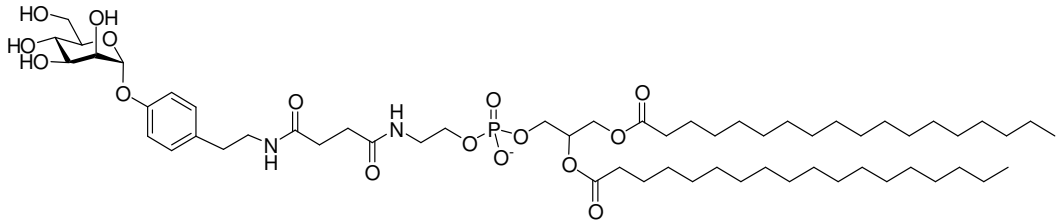

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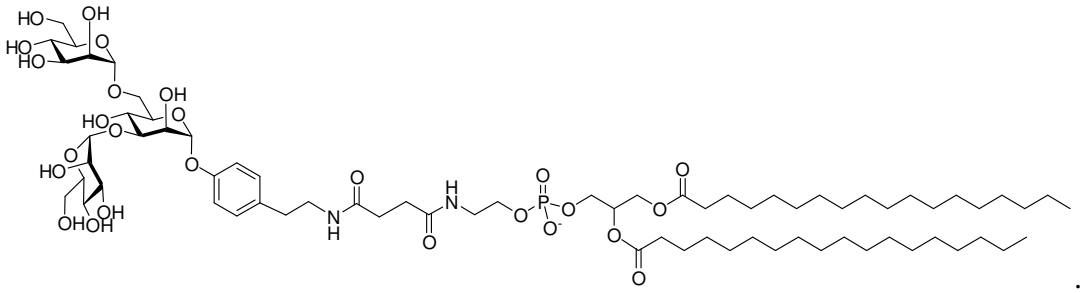


13. The lipid nanoparticle of any one of claims 1 to 12, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof.

14. The lipid nanoparticle of claim 13, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxyhexyl)amino]octanoate (SM-102^{TM}), (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315^{TM}, Pfizer), or a combination thereof.

15. The lipid nanoparticle of claim 13 or claim 14, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

16. The lipid nanoparticle of claim 15, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

17. The lipid nanoparticle of claim 15 or claim 16, wherein the cholesterol or the derivative thereof is a cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

18. The lipid nanoparticle of any one of claims 15 to 17, wherein the PEG-lipid is DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-

amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof.

19. The lipid nanoparticle of any one of claims 1 to 18, wherein the membrane encapsulates a payload, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

20. The lipid nanoparticle of claim 19, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

21. The lipid nanoparticle of claim 19 or claim 20, wherein the payload is a first payload, and the membrane further encapsulates a second payload, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof, and the first payload and the second payload are different.

22. The lipid nanoparticle of any one of claims 1 to 21, having a diameter of 0.01 to 5 microns.

23. A formulation, comprising the lipid nanoparticle of any one of claims 1 to 22.

24. The formulation of claim 23, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.

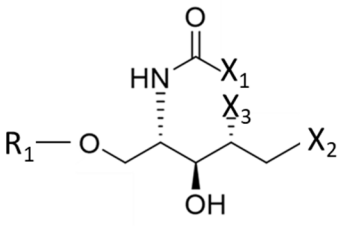
25. The formulation of claim 23 or claim 24, wherein the lipid nanoparticle is a first lipid nanoparticle, and the formulation further comprises a second lipid nanoparticle, further wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.

26. The formulation of any one of claims 23 to 25, further comprising an excipient, an adjuvant, or a combination thereof, 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; and/or the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, α-galactosylceramide, Aluminum salt, Squalene, MF59, or QS-21, aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), mixed aluminum

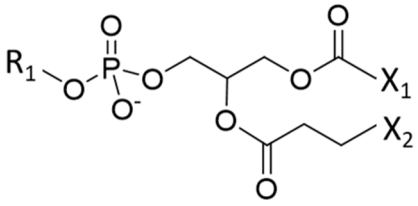
salts, Freund's complete adjuvant, Freund's incomplete adjuvant, AS03 (GlaxoSmithKline), MF59 (Seqirus), CpG 1018 (Dynavax), or a combination thereof.

27. A kit when used for preparing a lipid nanoparticle, comprising:

a first reagent, comprising a compound having Formula 1 or Formula 2:



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; and

a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.

28. The kit of claim 27, wherein 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.

29. The kit of claim 27 or claim 28, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

30. The kit of claim 29, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

31. The kit of claim 29 or claim 30, wherein the cholesterol or a derivative thereof is a cholesterol, campesterol, beta-sitosterol, brassicasterol, ergosterol, dehydroergosterol, stigmasterol, fucosterol, DC-cholesterol HCl, OH-Chol, HAPC-Chol, MHAPC-Chol, DMHAPC-Chol, DMPAC-Chol, cholesteryl chloroformate, GL67, cholesteryl myristate, cholesteryl oleate, cholesteryl nervonate, LC10, cholesteryl hemisuccinate, (3β,5β)-3-hydroxycholan-24-oic acid, alkyne cholesterol, 27-alkyne cholesterol, E-cholesterol alkyne, trifluoroacetate salt (Dios-Arg, 2H-Cho-Arg, or Cho-Arg), or a mixture thereof.

32. The kit of any one of claims 29 to 31, wherein the PEG-lipid is DMG-PEG, DSG-PEG, mPEG-DPPE, DOPE-PEG, mPEG-DMPE, mPEG-DOPE, DSPE-PEG-amine, DSPE-PEG, mPEG-DSPE, PEG PE, m-PEG-Pentacosadiynoic acid, bromoacetamido-PEG, amine-PEG, azide-PEG, or a mixture thereof.

33. The kit of any one of claims 27 to 32, further comprising a payload, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

34. The kit of claim 33, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

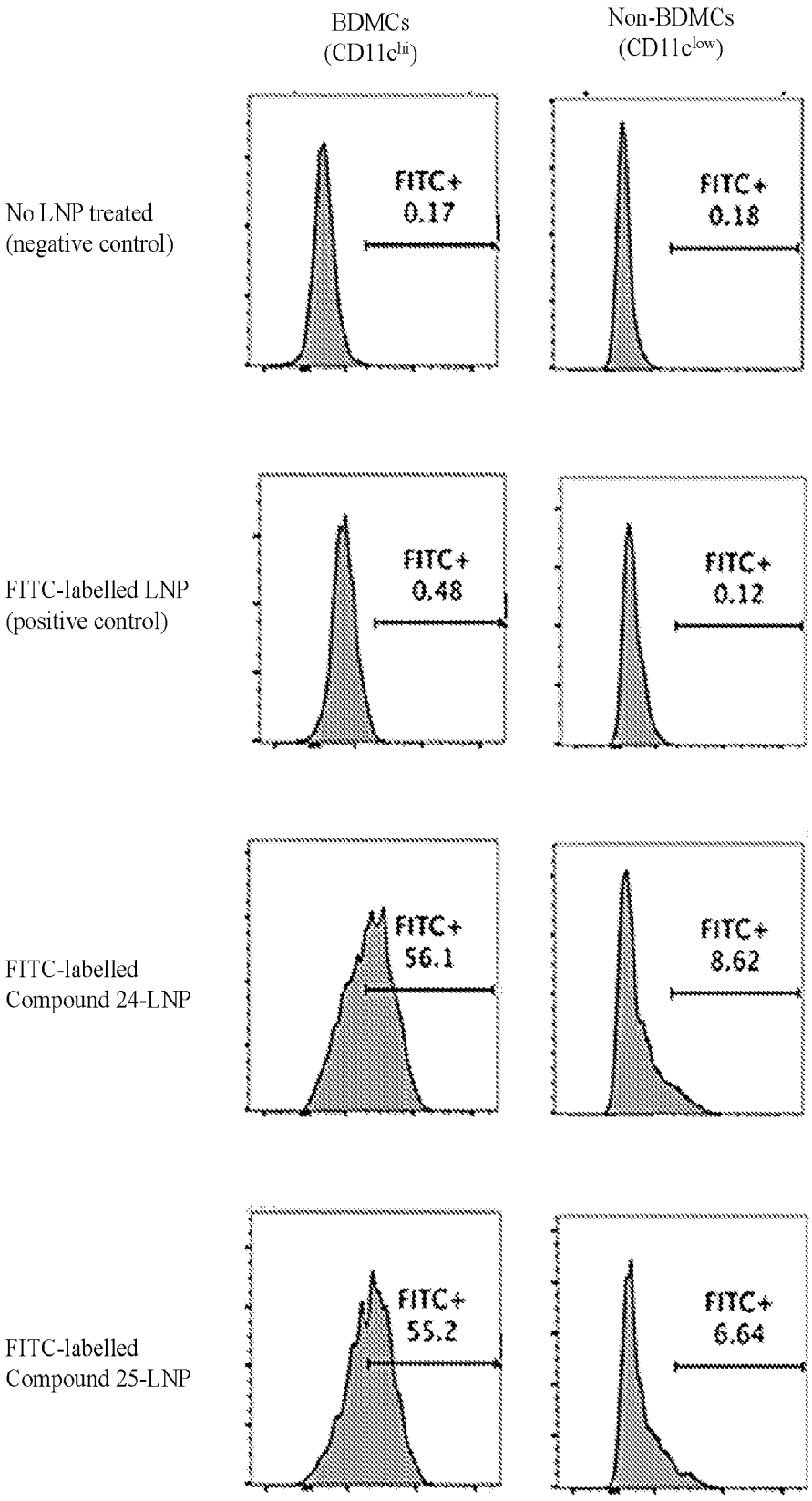


FIG. 1

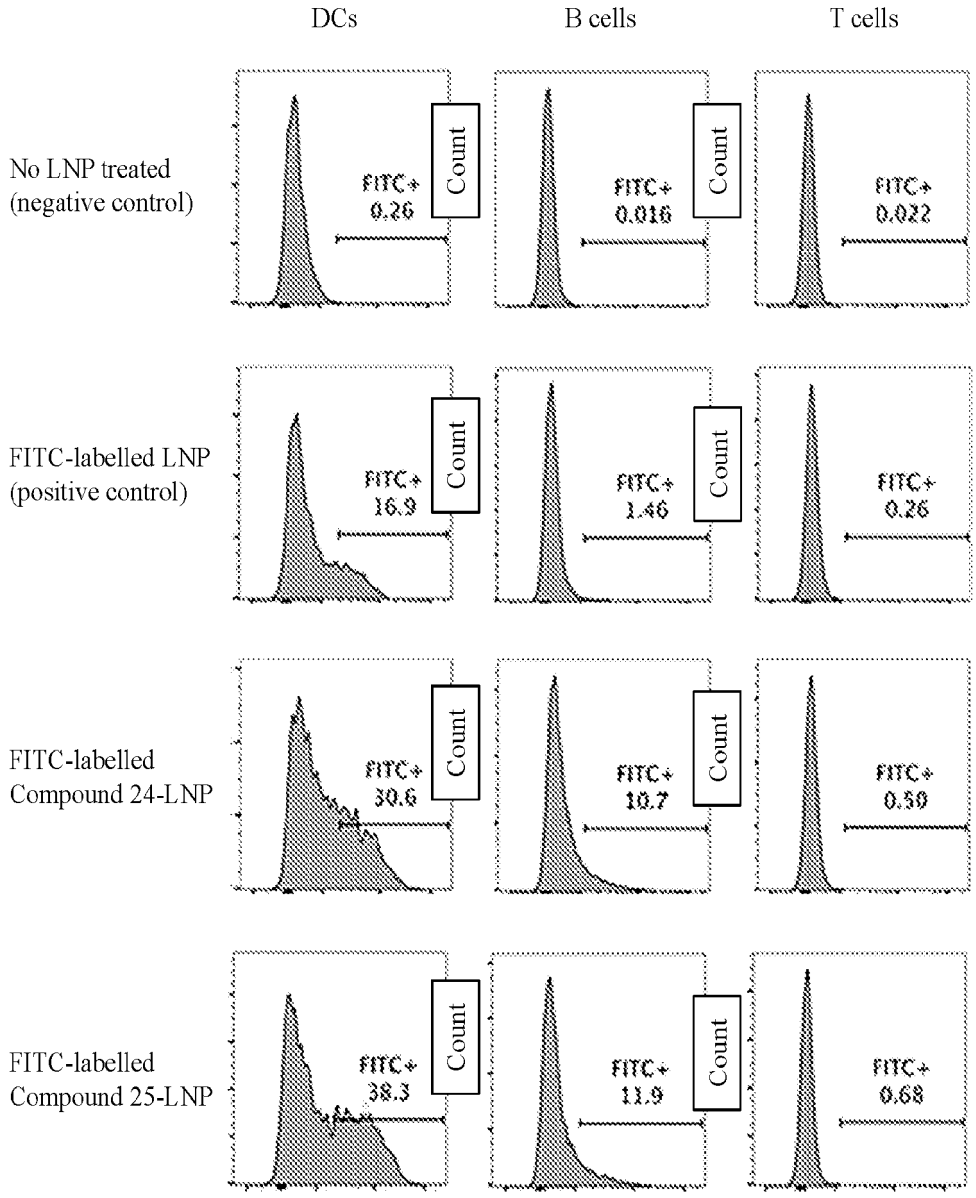


FIG. 2

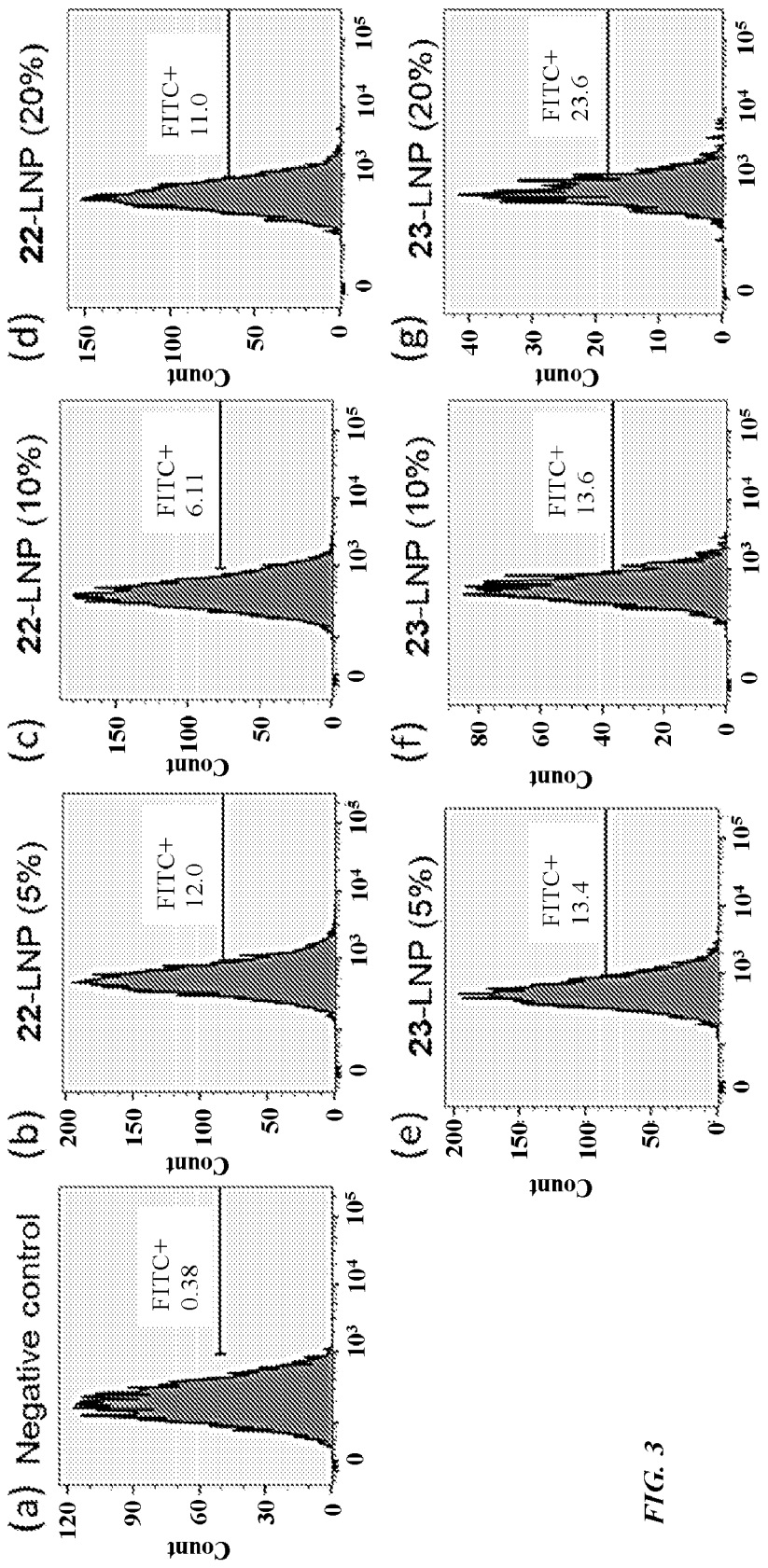


FIG. 3

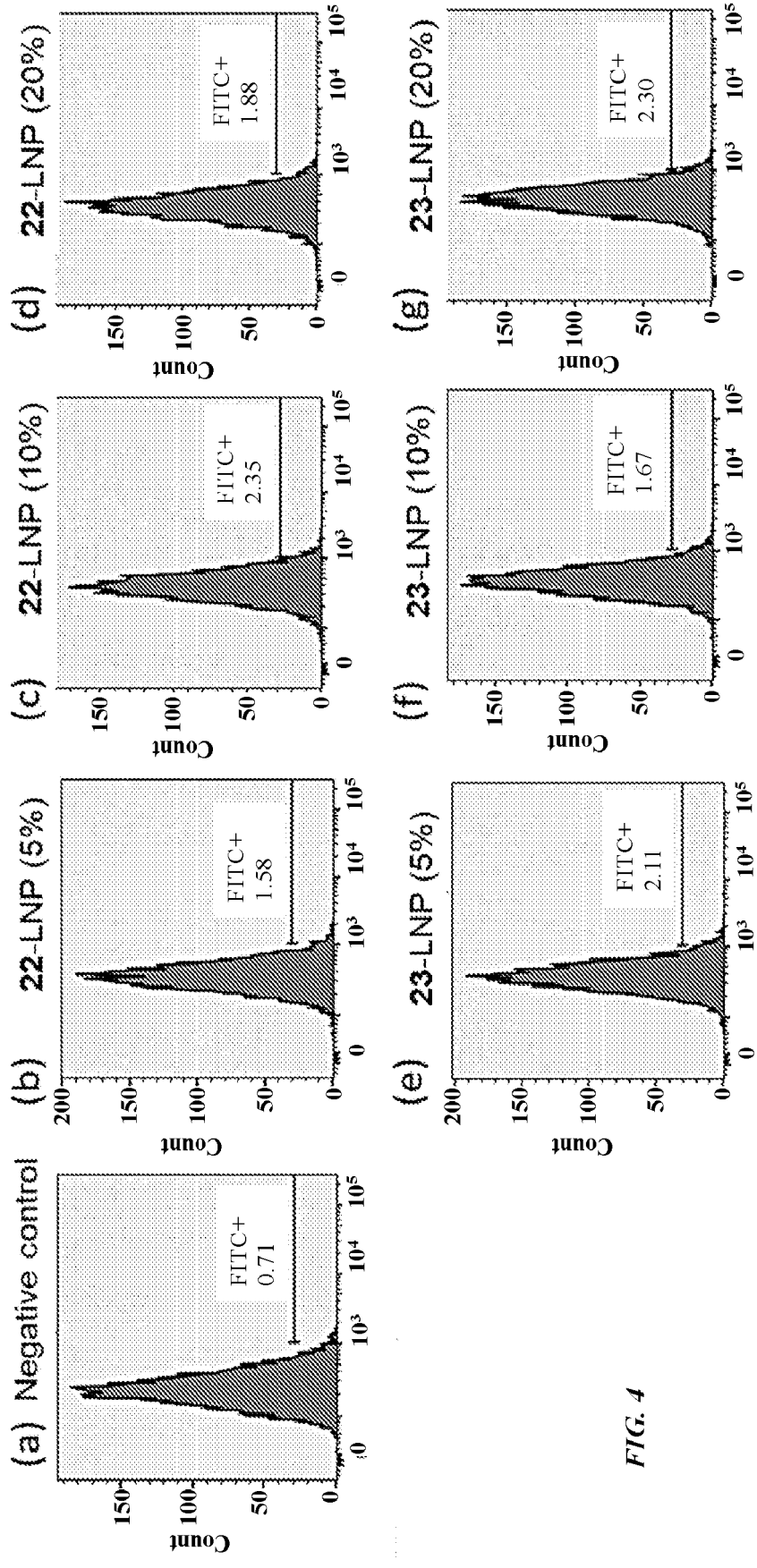


FIG. 4

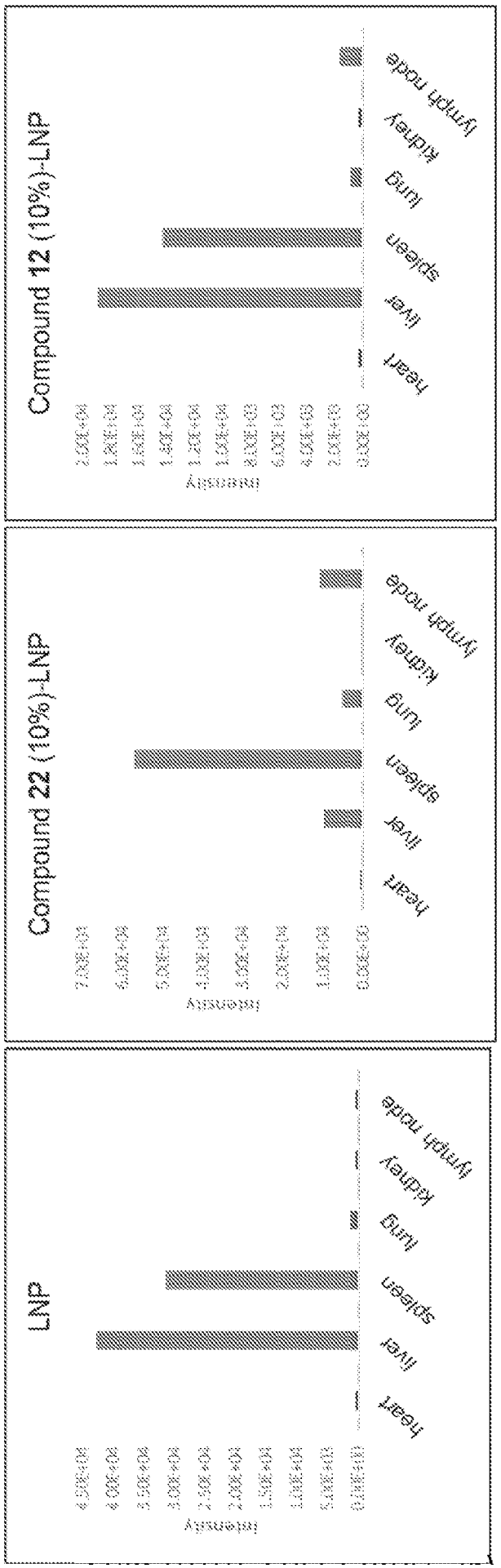


FIG. 5

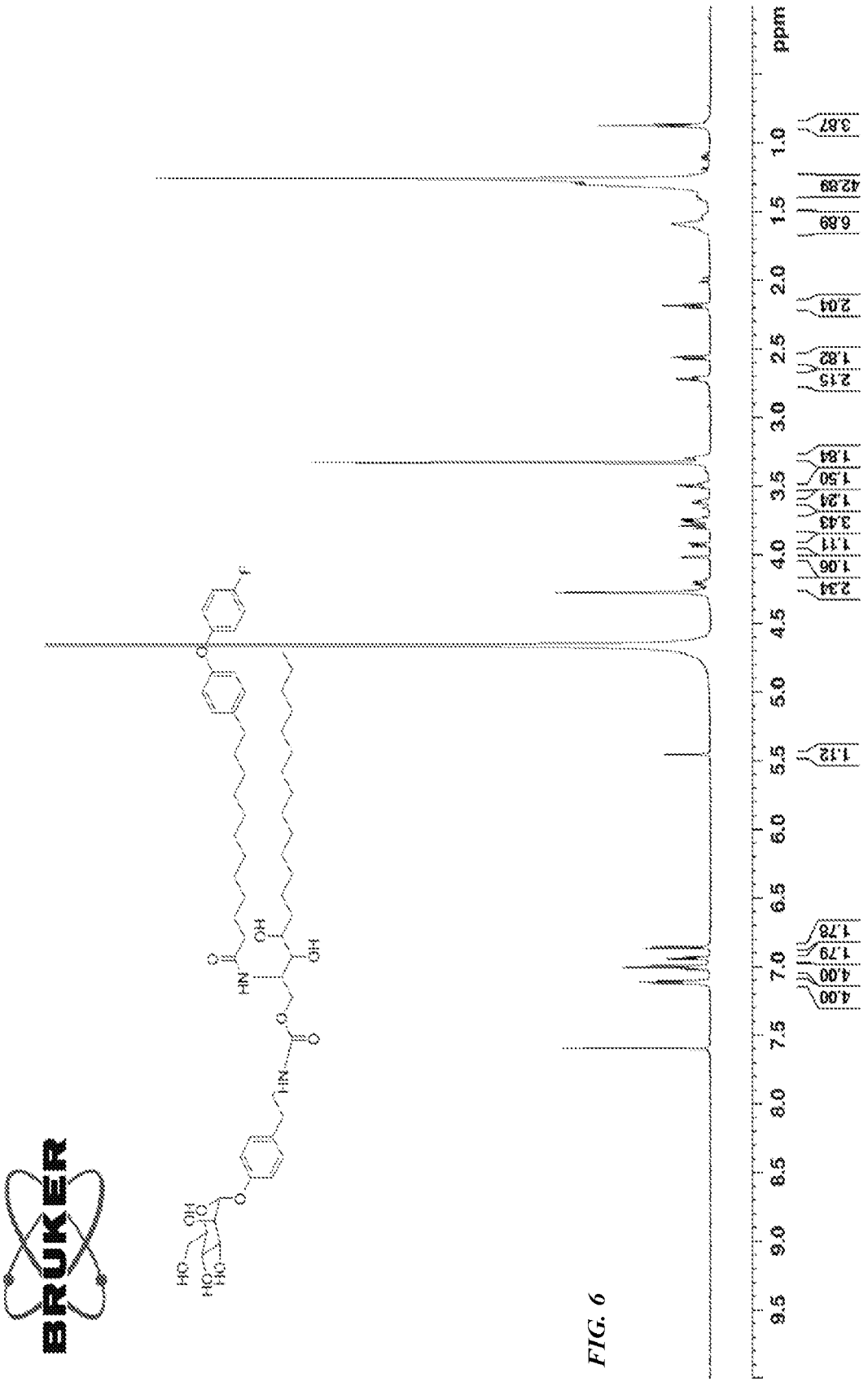


FIG. 6

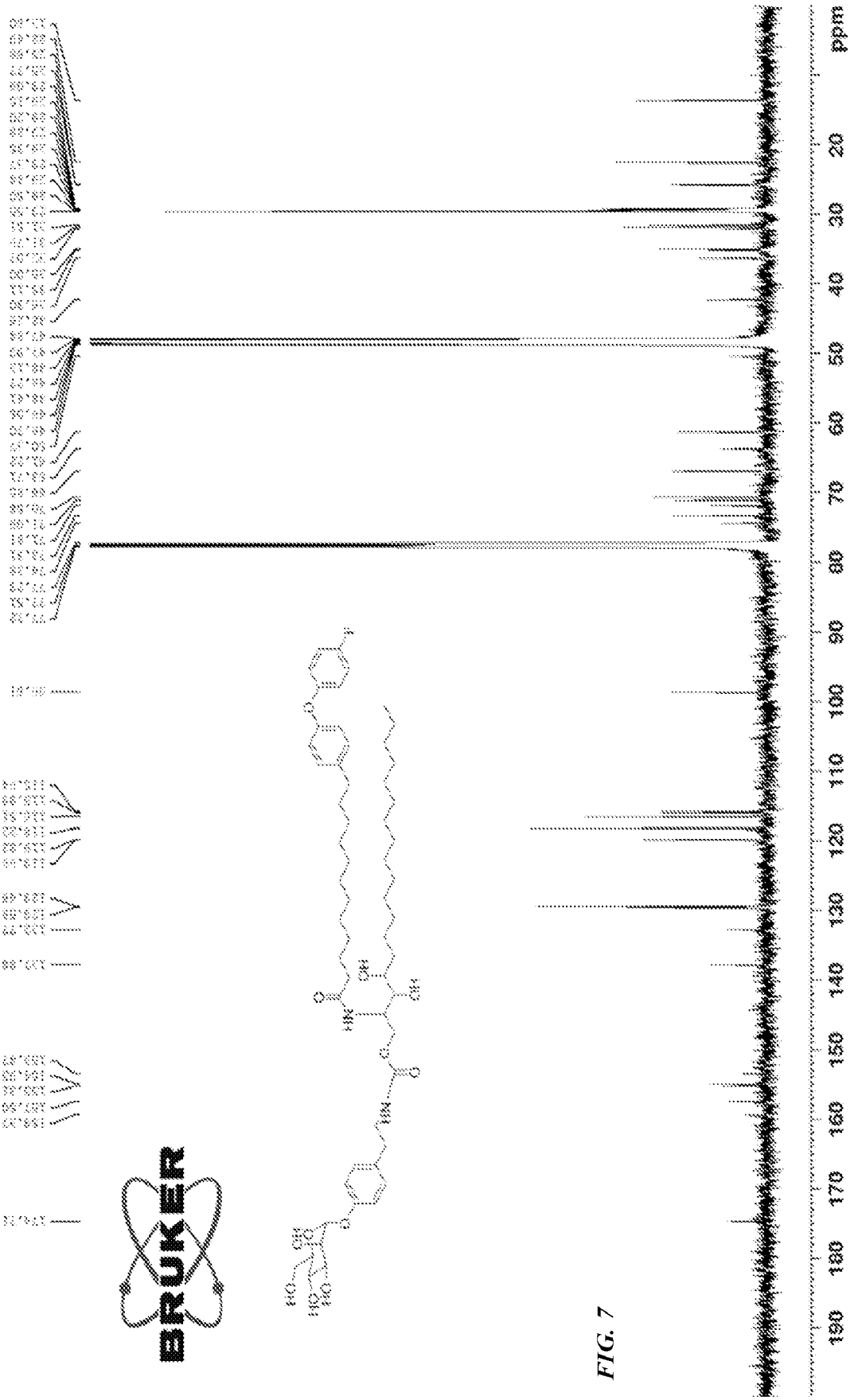


FIG. 7

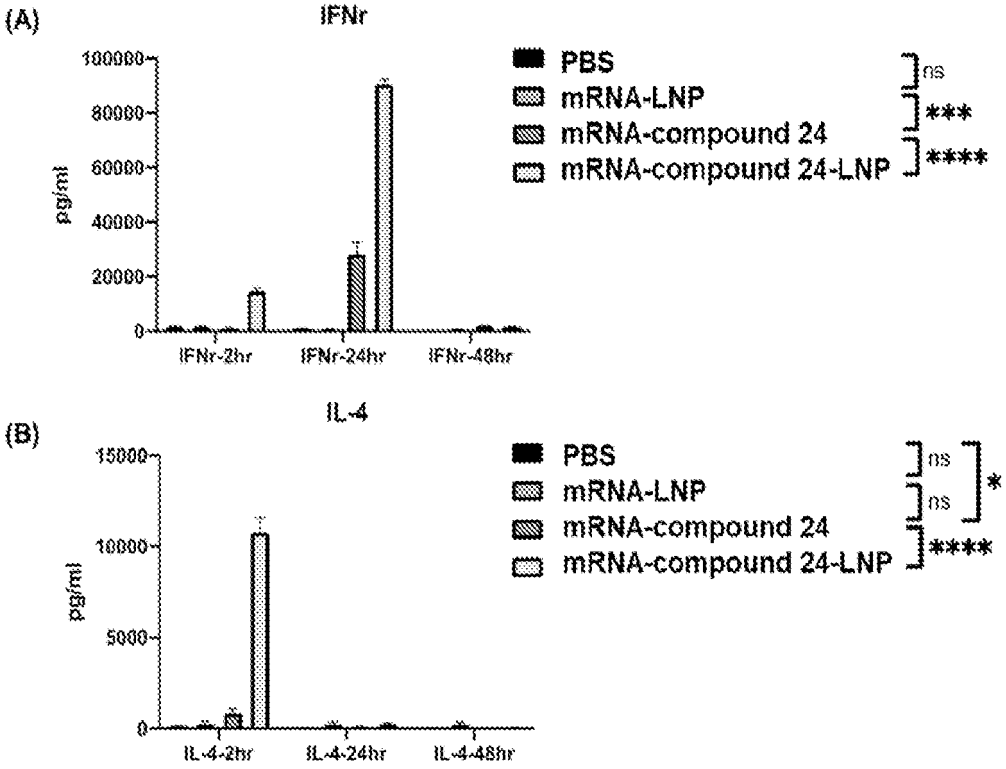


FIG. 8

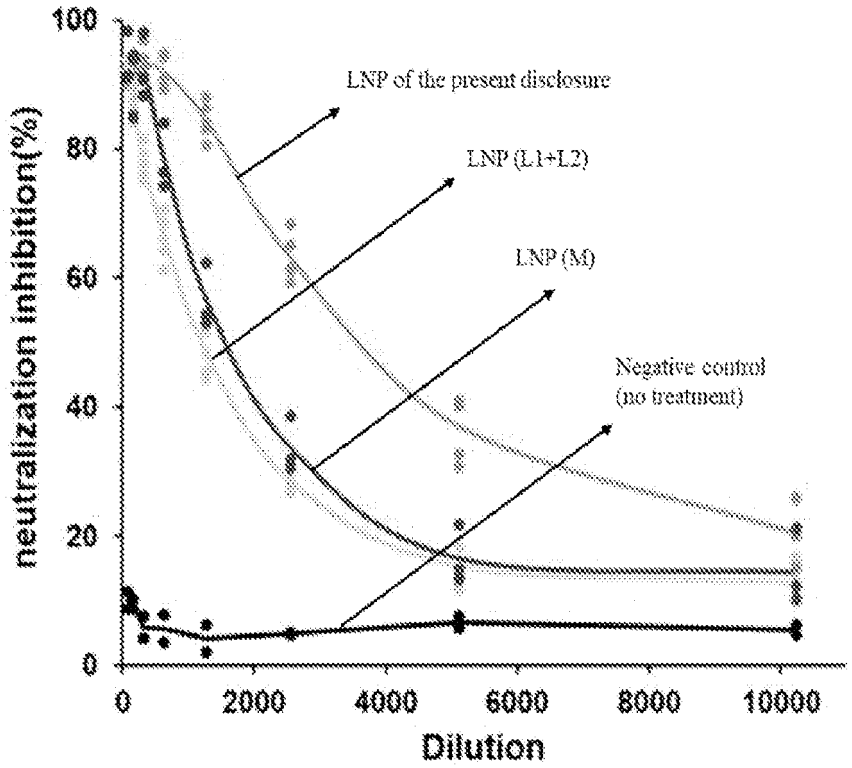


FIG. 9

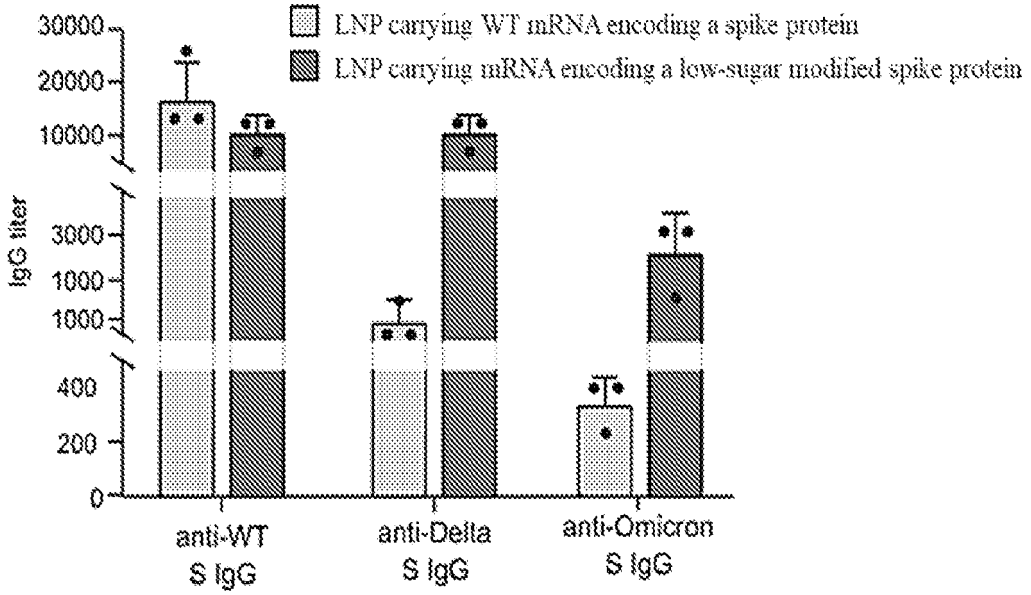


FIG. 10

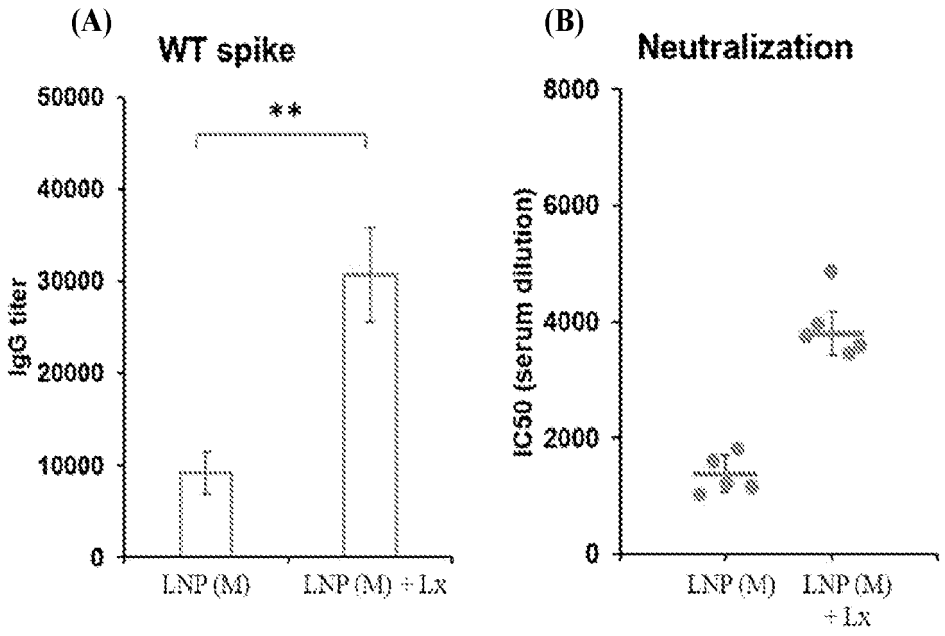
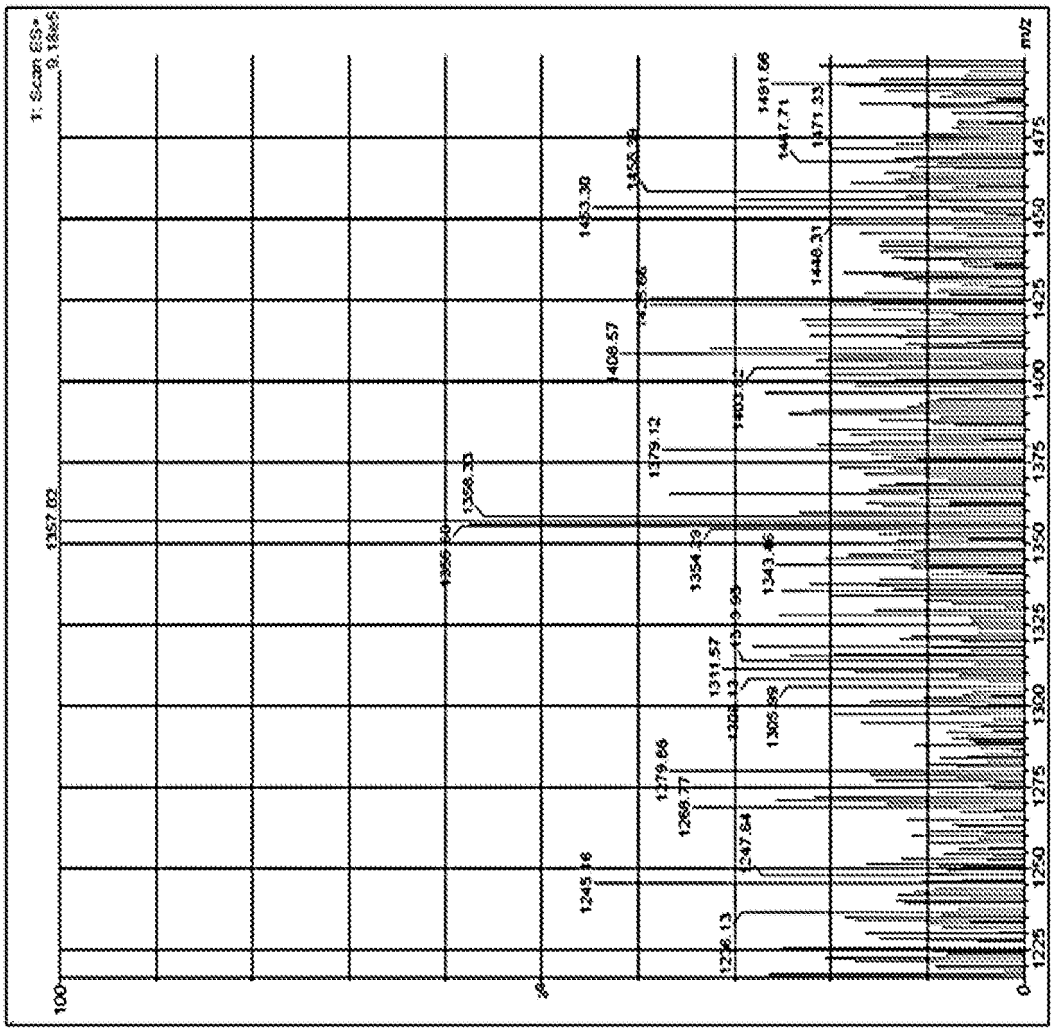


FIG. 11



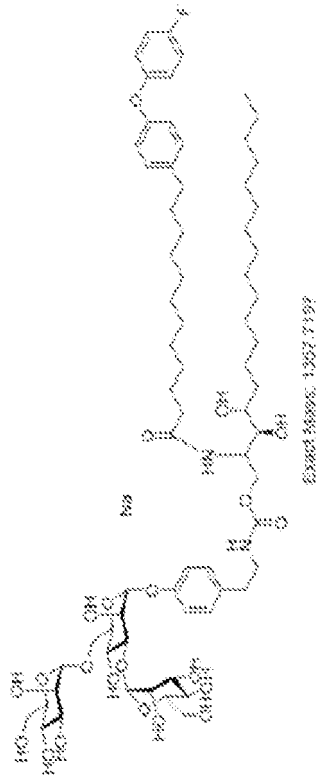


FIG. 12