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(54) Title: METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING VACCINES

(57) Abstract: The present disclosure provides novel compounds, methods, and cell targeting mRNA vaccine formulations for targeted delivery, such as delivery to dendritic cells. The compound and formulation provided herein are designed to have a targeting moiety configured to provide selective delivery features specific for dendritic cells and a lipid tail for incorporated into the bilayer membrane of the formed lipid nanoparticle.

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METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING VACCINES

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. 63/588,932, filed on October 9, 2023, U.S. Provisional Patent Applications No. 63/549,343, filed on February 2, 2024, 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 NANO-DELIVERY." The entireties of the aforementioned applications are incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII XML format under Rule ST.26 and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 5, 2024, is named A1000-01100PCT-3_20240405_SeqListing.xml and is 168 bytes in size.

FIELD

[0003] The instant disclosure relates to novel vaccine formulations for targeted nano-delivery. Specifically, the disclosure relates to compositions and methods directed to novel bi-functional nanoparticle vaccine formulations capable of selectively delivering a payload to a desired region in a tissue or a specific cell type, including dendritic cells.

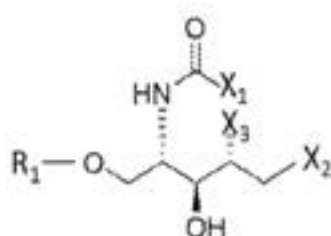
BACKGROUND OF THE INVENTION

[0004] 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

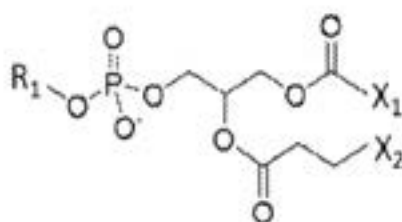
manufacturing of the lipid nanoparticles can be costly, and most importantly, the lipid nanoparticles generally cannot deliver the mRNA molecules with much localized selectivity. Therefore, there is an unmet need for novel vaccine nanoparticle formulations with selective delivery functionality.

BRIEF SUMMARY OF THE INVENTION

[0005] One aspect of the present disclosure is directed to a bi-functional compound for forming a lipid nanoparticle suitable for pharmaceutical vaccine 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.

[0006] 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.

[0007] One aspect of the present disclosure is directed to a dendritic cell targeting vaccine comprising 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.

[0008] One aspect of the present disclosure is directed to a bi-functional targeting nanocarrier vaccine composition/formulation, which comprises the lipid nanoparticle of the present disclosure.

[0009] 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.

[0010] One aspect of the present disclosure is directed to a method of targeted immunogenic 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 vaccine 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.

[0011] 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 vaccine 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 an immunogenic/therapeutic agent or derivatives an immunogenic/therapeutic agent.

[0012] 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 vaccine 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 derivatives of an immunogenic biomolecule.

[0013] In certain embodiments of the bi-functional molecule and vaccine 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

[0014] 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.).

[0015] 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, 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.).

[0016] 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).

[0017] 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 targeting 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).

[0018] 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.

[0019] FIG. 6 shows the ^1H NMR spectrum of compound 12 of the present disclosure.

[0020] FIG. 7 shows the ^{13}C NMR spectrum of compound 12 of the present disclosure.

[0021] FIG. 8 presents bar charts showing the IFN γ (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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] FIG. 12 shows the LCMS spectrum of compound 21 of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

[0026] 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

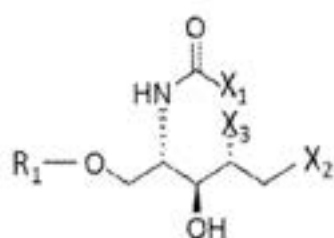
biocompatibility and biodegradability. A typical liposome has a bilayer structure formed by phospholipids due to their amphipathic properties. The bilayer structure (i.e., a bilayer membrane) encloses an inner space, which can encapsulate hydrophilic molecules, while the bilayer structure itself can 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.

[0027] 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.

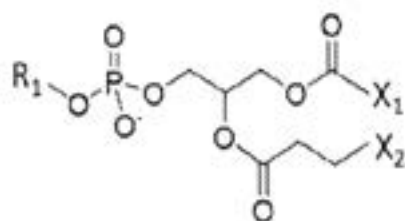
[0028] **TARGETING LIPID COMPOUND**

[0029] 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.

[0030] 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.

[0031] *R₁ group*

[0032] *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 receptor on a target cell. For example, the R_1 group might be configured to target the DC-SIGN of a dendritic cell.

[0033] 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.

[0034] 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- α 2,3-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-2, and the glycosyl group can comprise 9-Biphenyl Neu5Ac- α 2,6-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-5/E, and the glycosyl group can comprise Neu5Ac- α 2,3-Gal-GlcNAc.

[0035] 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_{1-6} alkyl, halide, or C_{1-6} 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.

[0036] *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.

[0037] *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.

[0038] 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 (OCH₂CH₂) subunits. In some embodiments, the PEG moiety can be a linear, branched, or star structure.

[0039] *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.

[0040] In some embodiments, the R₁ group is a substituted glycosyl group. The glycosyl group might comprise 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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).

[0041] 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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.

[0042] 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof. For example, the substituted benzene ring can be a phenol group.

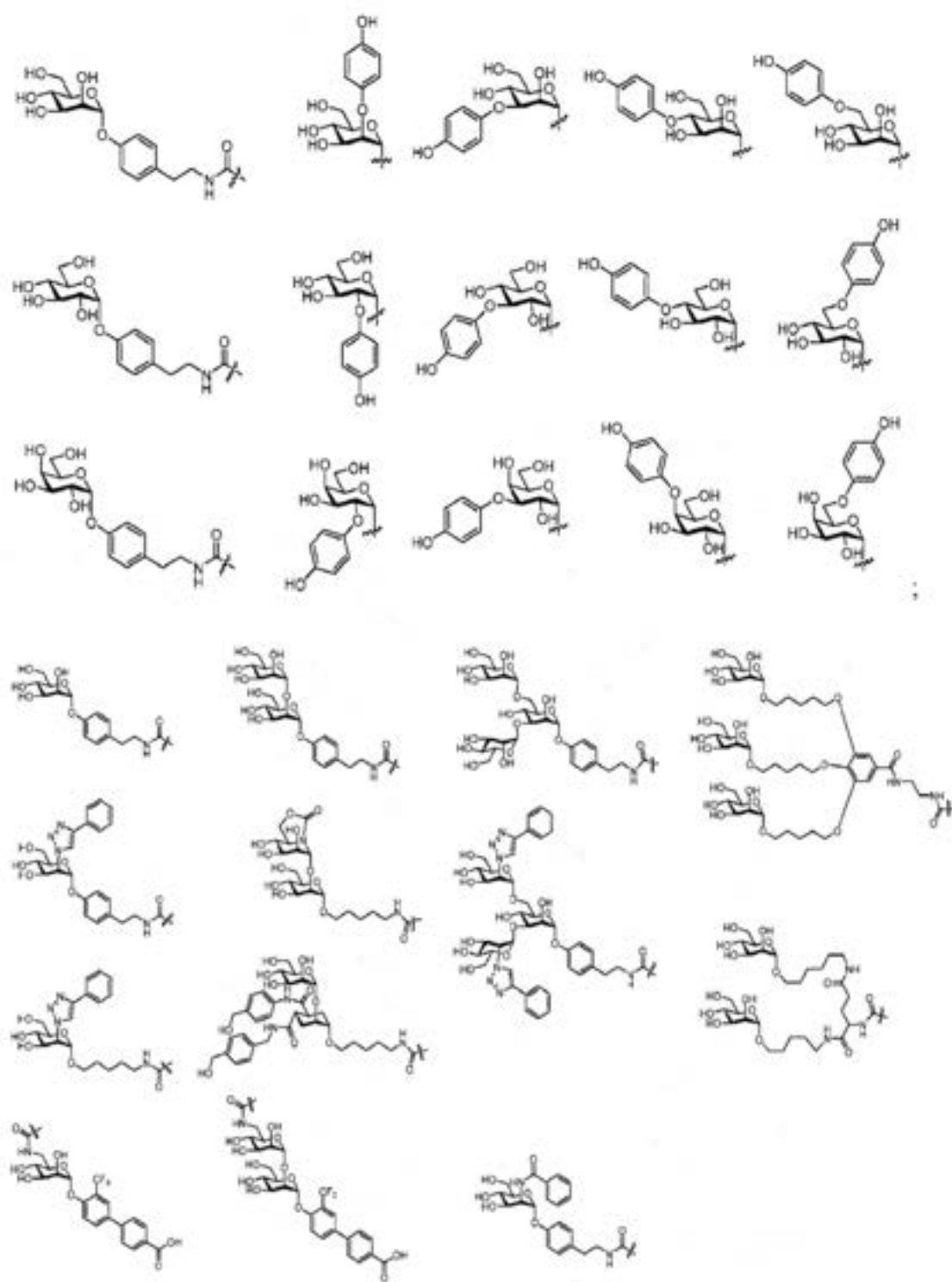
[0043] In some embodiments, the R_1 group is a mono-mannoside substituted with 1 to 6 substituents, and each substituent can be C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof,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

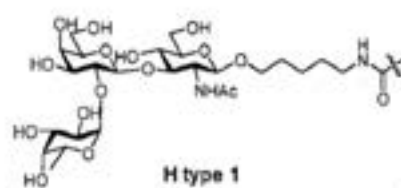
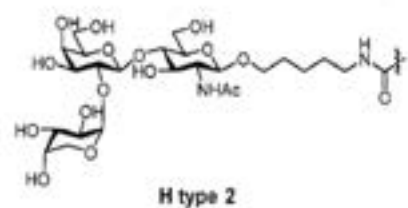
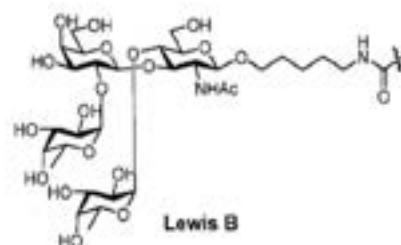
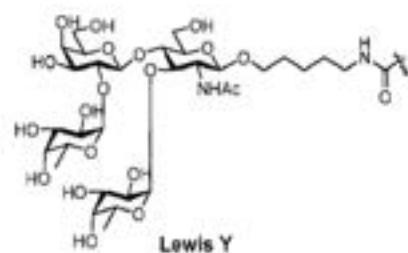
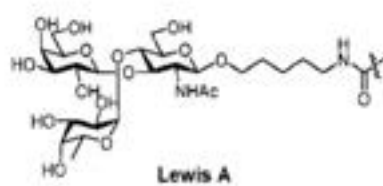
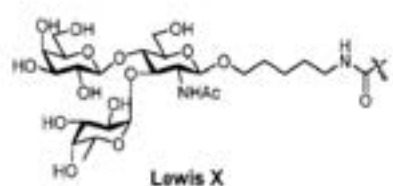
[0044] In some embodiments, the R_1 group comprises a first mannoside and a second mannoside. Each of the first mannoside and the second mannoside is independently substituted with 1 to 6 substituents, and each substituent can be C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

[0045] *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.

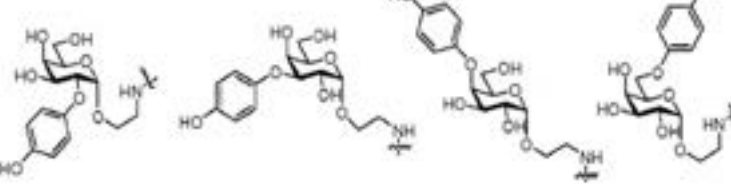
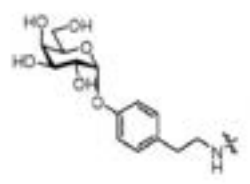
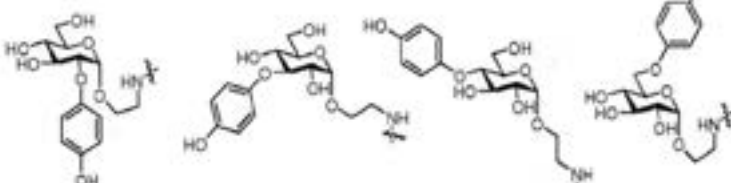
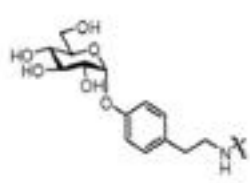
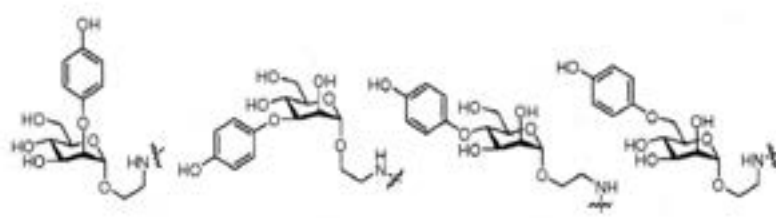
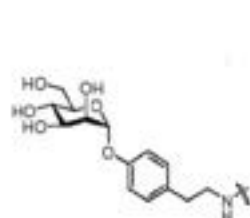
[0046] In some other embodiments, the K_D at pH 5 can be 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1250, 1500, 1750, or 2000 nM, or any range defined by the foregoing endpoints, such as, 1 to 2000, 1 to 1500, 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.

[0047] *Examples.* In some embodiments, the R₁ 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):

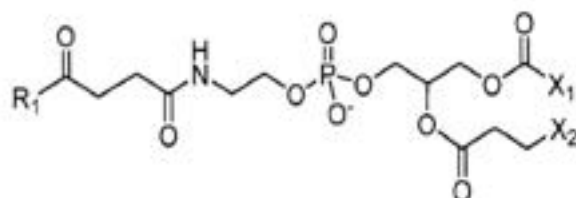




; and

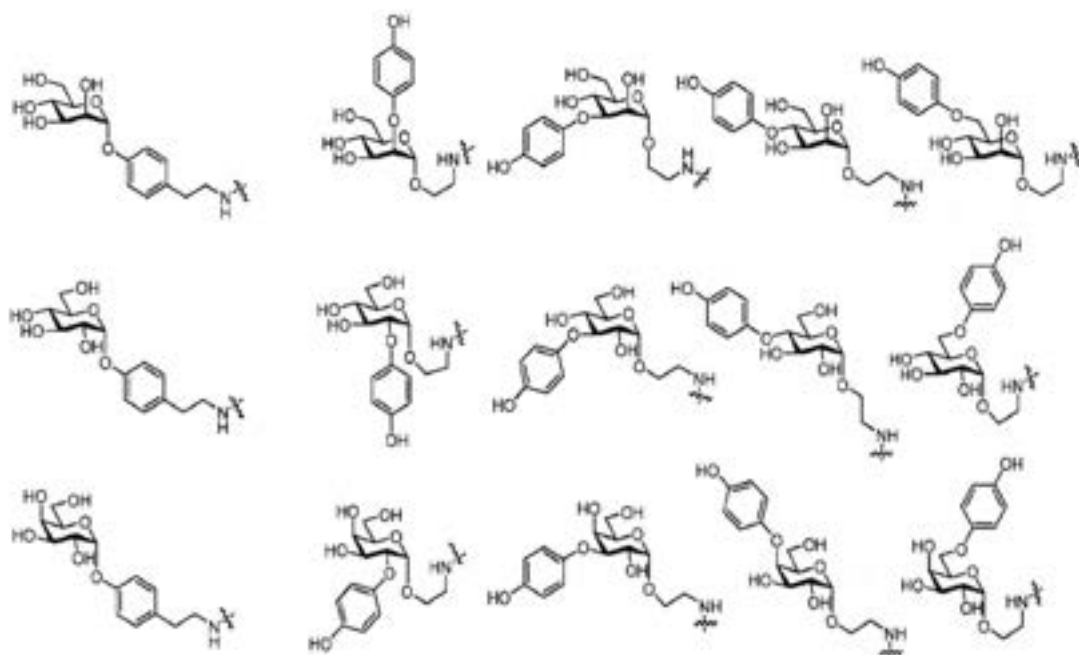


[0048] In some embodiments, the compound of the present disclosure has the structure shown in Formula 3:



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):



[0049] X_1 and X_2

[0050] 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.

[0051] 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.

[0052] 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.

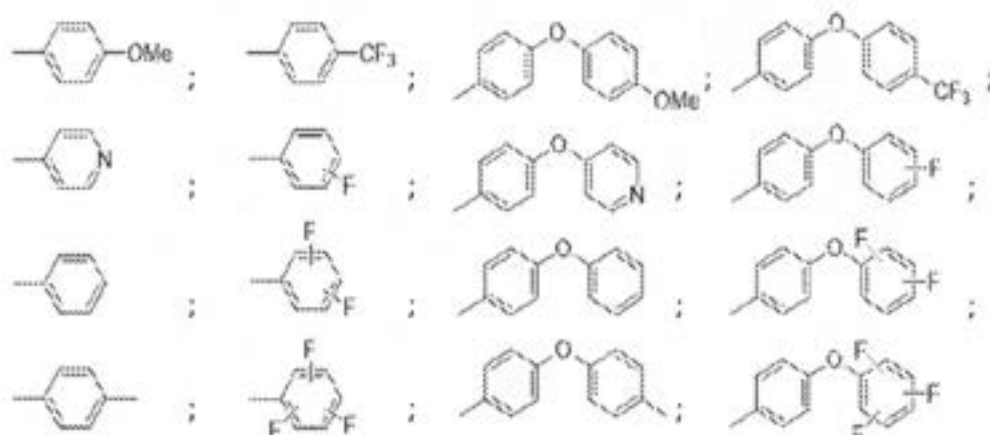
[0053] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{8-30} alkyl, C_{8-30} alkenyl, C_{8-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)_nX_4$, n is 8 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided 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.

[0054] 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.

[0055] 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 .

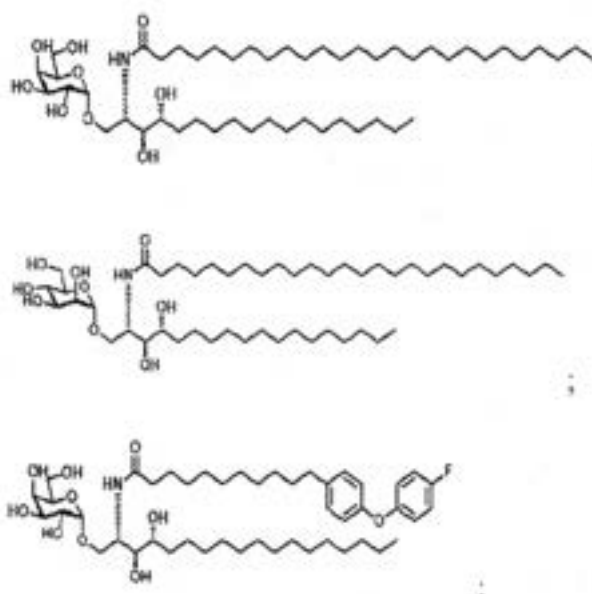
[0056] In some embodiments, X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

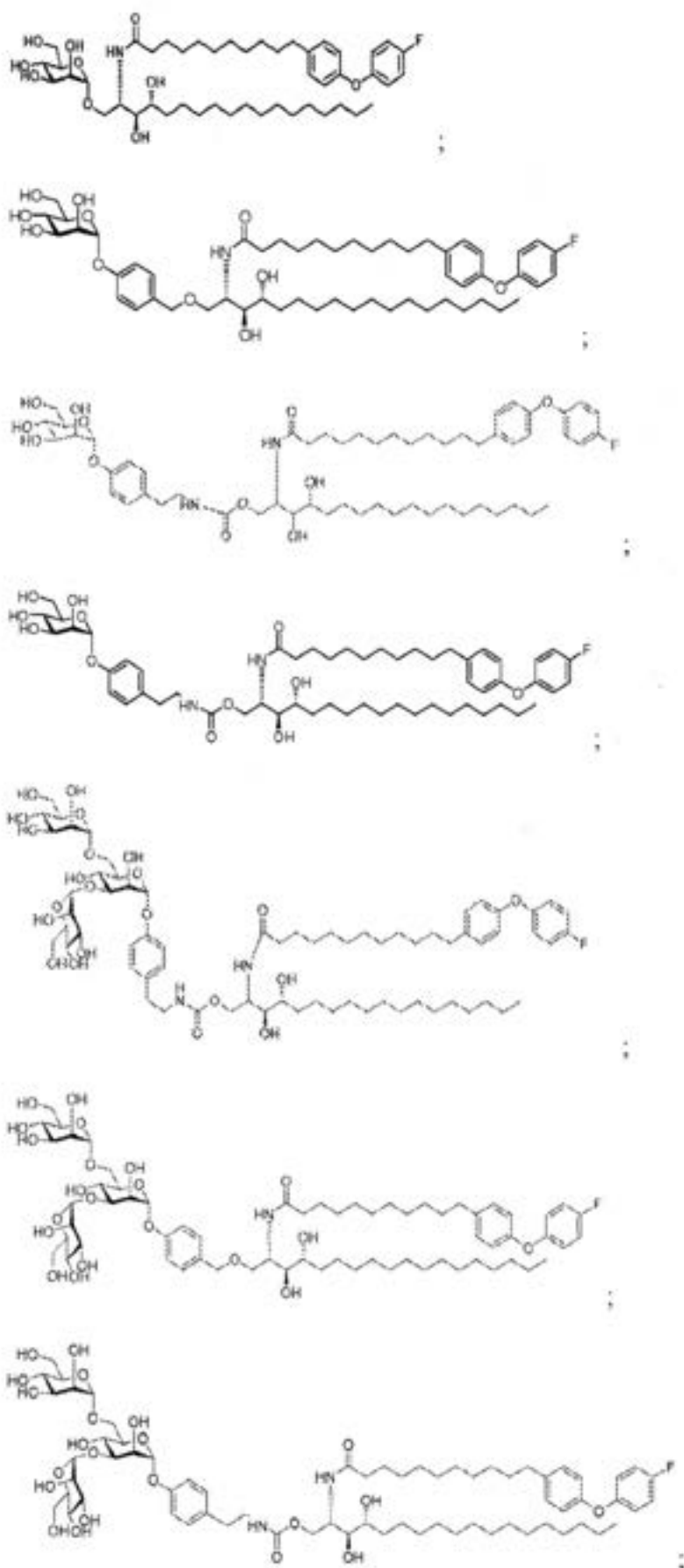
[0057] In certain embodiments, X_4 is selected from the group consisting of:

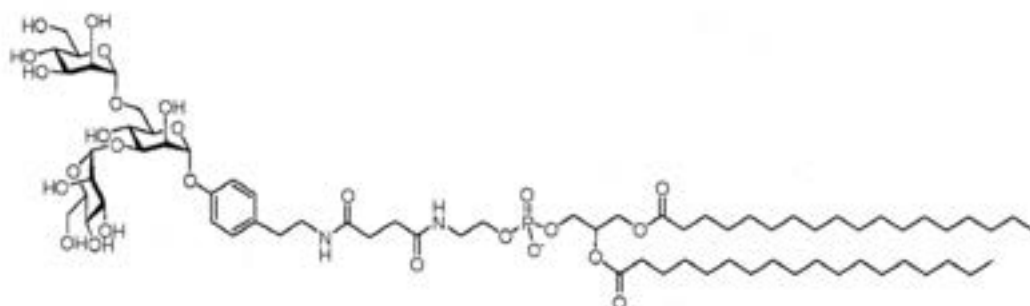
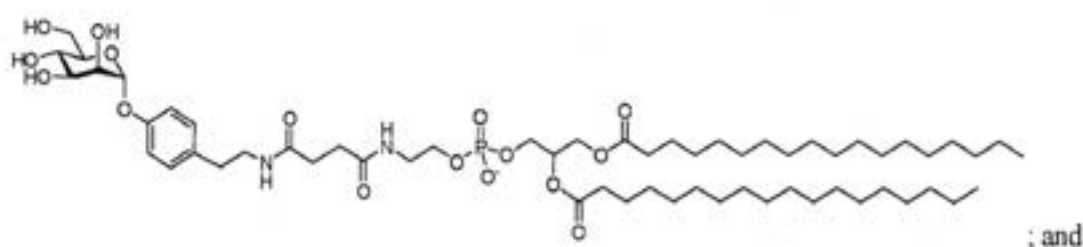
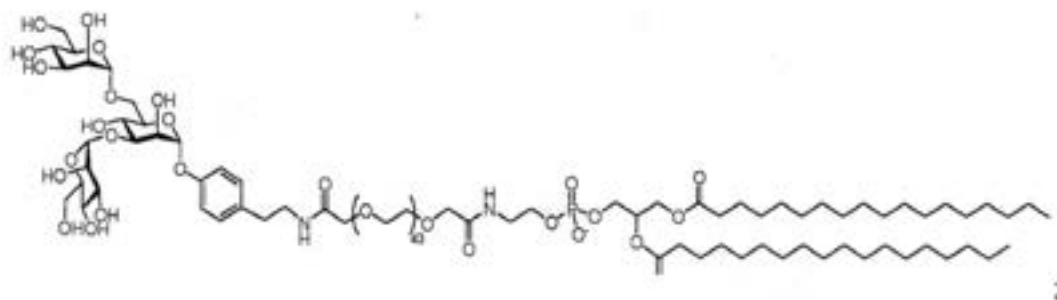
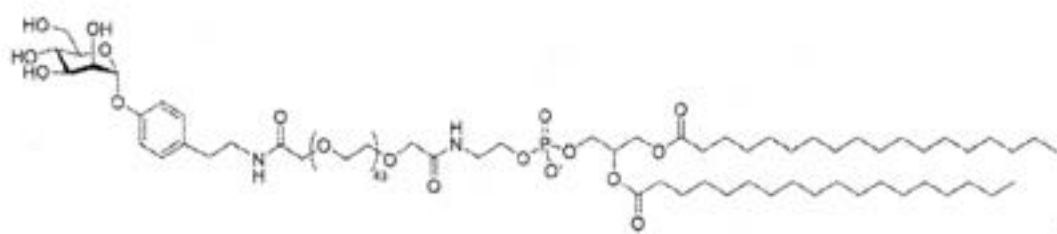


[0058] Exemplary Compound of the Present Disclosure

[0059] 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).







[0060] **COMPOSITION FOR FORMING A LIPID NANOPARTICLE**

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] *Ionizable lipid.*

[0066] 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-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-

hydroxybutyl)azanediy)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0067] *Helper lipid.*

[0068] A helper lipid is a lipid used to increase particle stability and fluidity of lipid nanoparticles. A helper 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.

[0069] 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.

[0070] *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.

[0071] *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, (3 β ,5 β)-3-hydroxycholelan-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.

[0072] *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 R₁ comprises a PEG moiety. In those circumstances, the composition does not need to comprise a PEG-lipid.

[0073] *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.

[0074] 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
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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%

[0075] LIPID NANOPARTICLE (LNP)

[0076] 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.

[0077] 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%.

[0078] 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.

[0079] 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%.

[0080] *Size of the LNP.*

[0081] 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.

[0082] *Zeta potential and molecular weight.*

[0083] Without wishing to be bound by theories, the zeta potential and molecular weight of an LNP may affect the cellular uptake of the 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.

[0084] *Payload*

[0085] 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.

[0086] 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.

[0087] 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 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.

[0088] 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'-O 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.

[0089] 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).

[0090] 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.

[0091] 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.

[0092] 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.

[0093] In some embodiments, the N/P ratio (positively-chargeable amine (nitrogen atom of the ionizable lipid, N = nitrogen) 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.

[0094] 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*.

[0095] *Payload encoding low-sugar immunogenic peptide (Low sugar universal vaccine).*

Without wishing to be bound by theories, it is documented that removing glycan shields of an immunogenic peptide can expose glycan shield-masked epitopes of the peptide, thereby increasing the antigenic presentation and the corresponding immune responses induced by the immunogenic peptide.

[0096] Compared with the wild-type spike proteins such as Wuhan strains and delta strains (such as SEQ ID NOs: 2, 16, 18, and 20), the modified spike protein described herein comprises one or more amino acid deletions or additions at N-linked glycosylation sequons (N-X-S/T) to eliminate N-linked glycan sequons. Alternatively, the modified spike protein described herein comprises one or more amino acid substitutions of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites.

[0097] *In the first aspect*, the exemplary mRNA of a coronavirus spike protein can be used as an exemplary coronavirus vaccine, which has a mutation of one or more glycosites in the receptor binding domain (RBD), the subunit 1 (S1) or the subunit 2 (S2) domain, or a variant thereof. When said mRNA is suitably formulated in the targeting LNP formulation of the present disclosure, it can be effectively used for dendritic cell targeting delivery. The mutation of CoV or a variant thereof, as described herein, can be deletion, addition, or substitution. In some embodiments, a coronavirus spike protein mRNA has one or more mutations of the glycosites in RBD, S1, or S2 with one or more replacements of N to Q or S/T to A, or a combination thereof. The mutation of the N-glycosites is to change the putative sequon N-X-S/T to Q-X-S/T and/or change S/T of the O-glycosite to A. The glycosites with N to Q replacement include, but are not limited to, the following:

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an S-(deg-RBD), which is an S protein with all 2 N-glycosites in RBD mutated from N to Q, and 2 O-glycosites mutated from S/T to A (such as SEQ ID NO: 4, 22, 24, or 26);

an S-(deg-S2), which is an S protein with all 9 glycosites in S2 mutated from N to Q (such as SEQ ID NO: 6, 28, 30, or 32);

an S-(deg-S2-1194), which is an S protein with 8 glycosites in S2 mutated from N to Q, except glycosite 1194 (such as SEQ ID NO: 8 or 34);

an S-(deg-RBD-801), which is an S protein with all 2 N-glycosites in RBD mutated from N to Q, and 2 O-glycosites mutated from S/T to A, and glycosite 801 mutated from N to Q (such as SEQ ID NO: 10 or 36);

an S-(deg-RBD-1194), which is an S protein with all 2 N-glycosites in RBD mutated from N to Q, and 2 O-glycosites mutated from S/T to A, and glycosite 1194 mutated from N to Q (such as SEQ ID NO: 12 or 38); and

an S-(deg-RBD-122-165-234), which is an S protein with all 2 N-glycosites in RBD mutated from N to Q, and 2 O-glycosites mutated from S/T to A, and glycosite 122, 165, and 234 mutated from N to Q (such as SEQ ID NO: 14 or 40).

[0098] In a further embodiment, the mRNA or DNA for S-(deg-RBD) has the sequence of SEQ ID NO: 3, 21, 23, or 25, the mRNA or DNA for S-(deg-S2) has the sequence of SEQ ID NO: 5, 27, 29, or 31, the mRNA or DNA for S-(deg-S2-1194) has the sequence of SEQ ID NO: 7 or 33, the mRNA or DNA for S-(deg-RBD-801) has the sequence of SEQ ID NO: 9 or 35, the mRNA or DNA for S-(deg-RBD-1194) has the sequence of SEQ ID NO: 11 or 37, the mRNA or DNA for S-(deg-RBD-122-165-234) has the sequence of SEQ ID NO: 13 or 39.

[0099] The present disclosure also provides a linear DNA comprising a promoter, 5' untranslated region, 3' untranslated region, expression plasmid with or without S-2P, and poly(A) tail signal sequence, wherein the putative sequon N-X-S/T is changed to Q-X-S/T and the O-glycosite was changed from S/T to A on the expression plasmid. In one embodiment, the S-2P expression plasmid comprises the S gene of SARS-CoV-2 encoding the pre-fusion state of the S having proline substitutions of K968 and V969.

[0100] In certain embodiments, the cell targeting formulation of the present disclosure can contain exemplary immunogenic peptides with glycan shields removed, which further can include, but are not limited to, an immunogenic peptide, comprising at least one amino acid sequence selected from a group

consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIQAG (SEQ ID NO: 46), GPKKSTNLVKNNK (SEQ ID NO: 47), NCDVVIGIV[N]NTVY (SEQ ID NO: 48), PELDSFKEELDKYFK[N]HTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NL[N]ESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.

[0101] In some embodiments, the cell-targeting formulation payload comprises an RNA encoding a modified protein comprising (1) one or more amino acid substitutions of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue; and/or (2) one or more amino acid substitutions of S/T to alanine (A) at the O-glycosites. In certain embodiments, the payload comprises an RNA encoding an immunogenic peptide, which comprises at least one amino acid sequence selected from a group consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIQAG (SEQ ID NO: 46), GPKKSTNLVKNNK (SEQ ID NO: 47), NCDVVIGIV[N]NTVY (SEQ ID NO: 48), PELDSFKEELDKYFK[N]HTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NL[N]ESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.

[0102] In some embodiments, the cell targeting formulation payload is a nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO: 01, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, or at least about 99%, 98%, 97%, 96%, 95% or 90% identity thereto; or the payload is configured to encode a peptide comprising an amino acid sequence set forth in SEQ ID NO: 02, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, or at least about 99%, 98%, 97%, 96%, 95% or 90% identity thereto.

[0103] In the second aspect, exemplary immunogenic peptides with glycan shields removed include, but are not limited to, an immunogenic peptide, comprising at least one amino acid sequence selected from a group consisting of: SSANNCTFEYVSQ (SEQ ID NO: 58), TESIVRFPNITNL (SEQ ID NO: 59), KPFERDISTEIQAG (SEQ ID NO: 60), GPKKSTNLVKNKC (SEQ ID NO: 61), TEVPVAIHADQ (SEQ ID NO: 62), RVYSTGSNVFQTR (SEQ ID NO: 63), RRARSVASQS (SEQ ID NO: 64), DPSKPSKRSF (SEQ ID NO: 65), FIKQYGDCLGDI (SEQ ID NO: 66), ENQKLIANQFNS (SEQ ID NO: 67), GKIQDSLSTA (SEQ ID NO: 68), NCDVVIGIVNNTVY (SEQ ID NO: 69), PELDSFKEELDKYFKNHTS (SEQ ID NO: 70), TSPDVLGDISGINA (SEQ ID NO: 71), VNIQKEIDRLNEVA (SEQ ID NO: 72), NLNESLIDLQ (SEQ ID NO: 73), and LGKYEQYIKWP (SEQ ID NO: 74).

[0104] In some embodiments, the cell-targeting formulation payload comprises an RNA encoding a modified protein comprising (1) one or more amino acid substitutions of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue; and/or (2) one or more amino acid substitutions of S/T to alanine (A) at the O-glycosites. In certain embodiments, the payload comprises an RNA encoding an immunogenic peptide, which comprises at least one amino acid sequence selected from a group consisting of: SSANNCTFEYVSQ (SEQ ID NO: 58), TESIVRFPNITNL (SEQ ID NO: 59), KPFERDISTEIQAG (SEQ ID NO: 60), GPKKSTNLVKNKC (SEQ ID NO: 61), TEVPVAIHADQ (SEQ ID NO: 62), RVYSTGSNVFQTR (SEQ ID NO: 63), RRARSVASQS (SEQ ID NO: 64), DPSKPSKRSF (SEQ ID NO: 65), FIKQYGDCLGDI (SEQ ID NO: 66), ENQKLIANQFNS (SEQ ID NO: 67), GKIQDSLSTA (SEQ ID NO: 68), NCDVVIGIVNNTVY (SEQ ID NO: 69), PELDSFKEELDKYFKNHTS (SEQ ID NO: 70), TSPDVLGDISGINA (SEQ ID NO: 71), VNIQKEIDRLNEVA (SEQ ID NO: 72), NLNESLIDLQ (SEQ ID NO: 73), and LGKYEQYIKWP (SEQ ID NO: 74).

[0105] In some embodiments, the cell-targeting formulation payload is configured to encode a peptide comprising an amino acid sequence set forth in SEQ ID NO: 53, 54, 55, 56, or 57, or at least about 99%, 98%, 97%, 96%, 95% or 90% identity thereto.

[0106] As described herein, the exemplary Low Sugar Universal Vaccine (LSUV) is also 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.

[0107] *Kit for Preparing a Lipid Nanoparticle.*

[0108] 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 cell targeting payload can be as those described herein.

[0109] *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.

[0110] **COMPOSITIONS/FORMULATIONS**

[0111] One aspect of the present disclosure is directed to a pharmaceutically acceptable vaccine composition comprising the targeting 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.

[0112] 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%

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(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.

[0113] 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.

[0114] 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.

[0115] 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).

[0116] 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.

[0117] *Combination compositions*

[0118] 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.

[0119] 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.

[0120] 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.

[0121] *Additional components of the composition*

[0122] 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.

[0123] 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.).

[0124] 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.

[0125] 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).

[0126] 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).

[0127] 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.

[0128] **METHODS OF USE**

[0129] 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.

[0130] *Methods of targeted payload delivery in a subject*

[0131] 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.

[0132] *Methods of preventing or treating a disease in a subject*

[0133] 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.

[0134] 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 R₁ 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.

[0135] 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.

[0136] 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.

[0137] *Methods of boosting an adaptive immune response*

[0138] 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.

[0139] 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.

[0140] 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.

[0141] *Administration*

[0142] 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.

[0143] *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.

[0144] *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.

[0145] 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).

[0146] 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.

[0147] 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.

[0148] 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%.

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] **DEFINITION**

[0154] 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.

[0155] 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 $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate.

[0156] 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.

[0157] 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.

[0158] 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.

[0159] 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.).

[0160] 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, C₁₋₆ 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.

[0161] 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, C₂₋₆ 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.

[0162] 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, C₂₋₆ 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.

[0163] As used herein, "cycloalkyl" refers to a nonaromatic cyclic, bicyclic, 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.

[0164] 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.

[0165] 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, cinnolyl, phthalazinyl, quinazolinyl, quinoxalinyl, triazinyl, indolyl, isoindolyl, indazolyl, indolizinyl, purinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, benzoxazolyl, benzothiazolyl, 1H-benzimidazolyl, imidazopyridinyl, benzothieryl, benzofuranyl, isobenzofuran and the like.

[0166] 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 —NH₂, —NHR, or —NR₂, where R is the same or different at each occurrence and can be an alkyl group or an aryl group.

[0167] 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.

[0168] 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. Dihalalkyl 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.

[0169] As used herein, unless otherwise specified, the term "heteroatom" refers to a nitrogen (N), oxygen (O), or sulfur (S) atom.

[0170] **EXAMPLE**

[0171] **Example 1: Synthesis of exemplary compounds of the present disclosure**

[0172] *Chemical Materials and Methods*

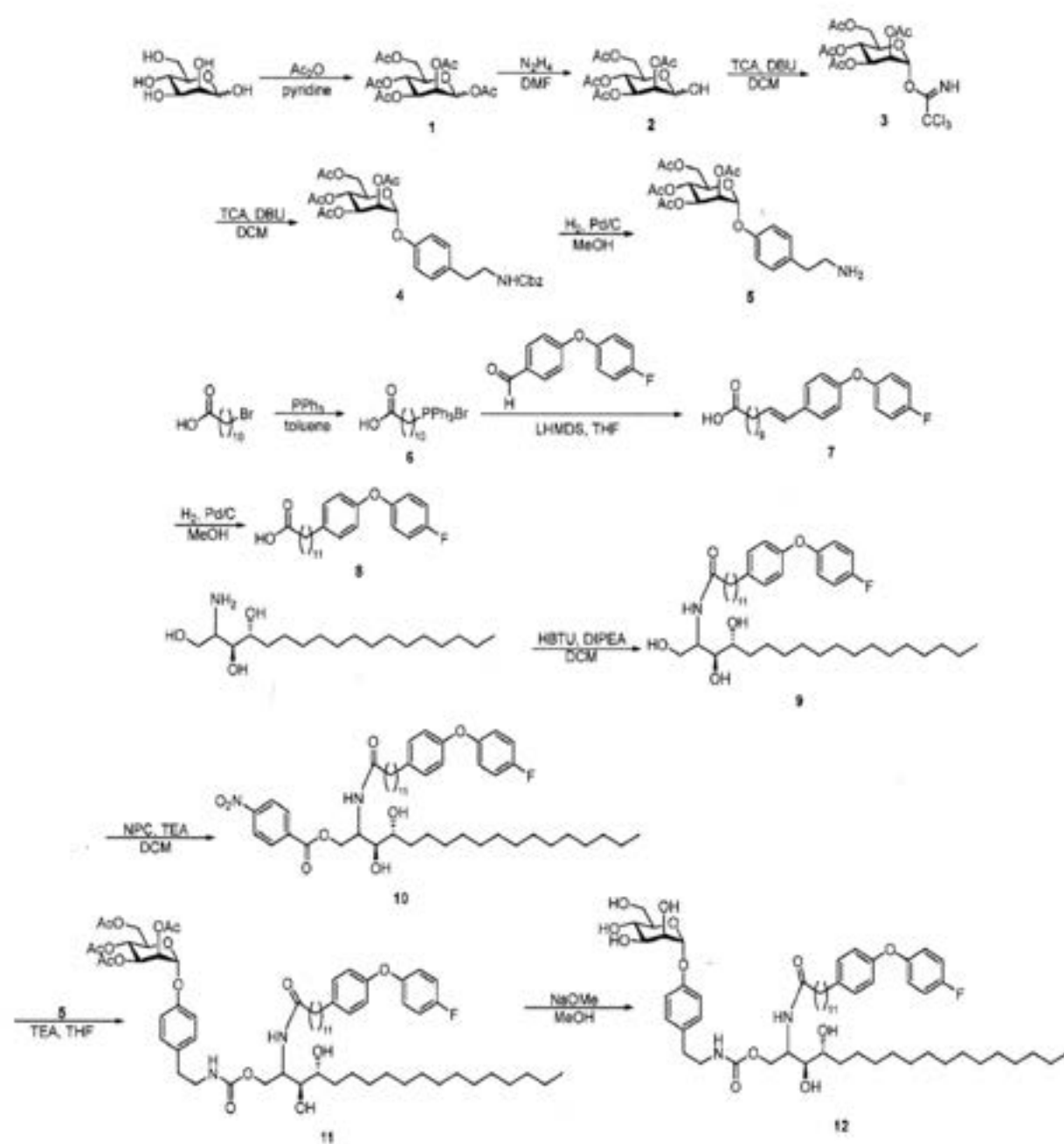
[0173] For chemical synthesis, all starting materials and commercially obtained reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry solvents. ^1H and ^{13}C NMR spectra were recorded on Bruker 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 ^1H 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. IMPACTTM 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.

[0174] The chemical materials and methods described herein apply to all examples described in the present disclosure.

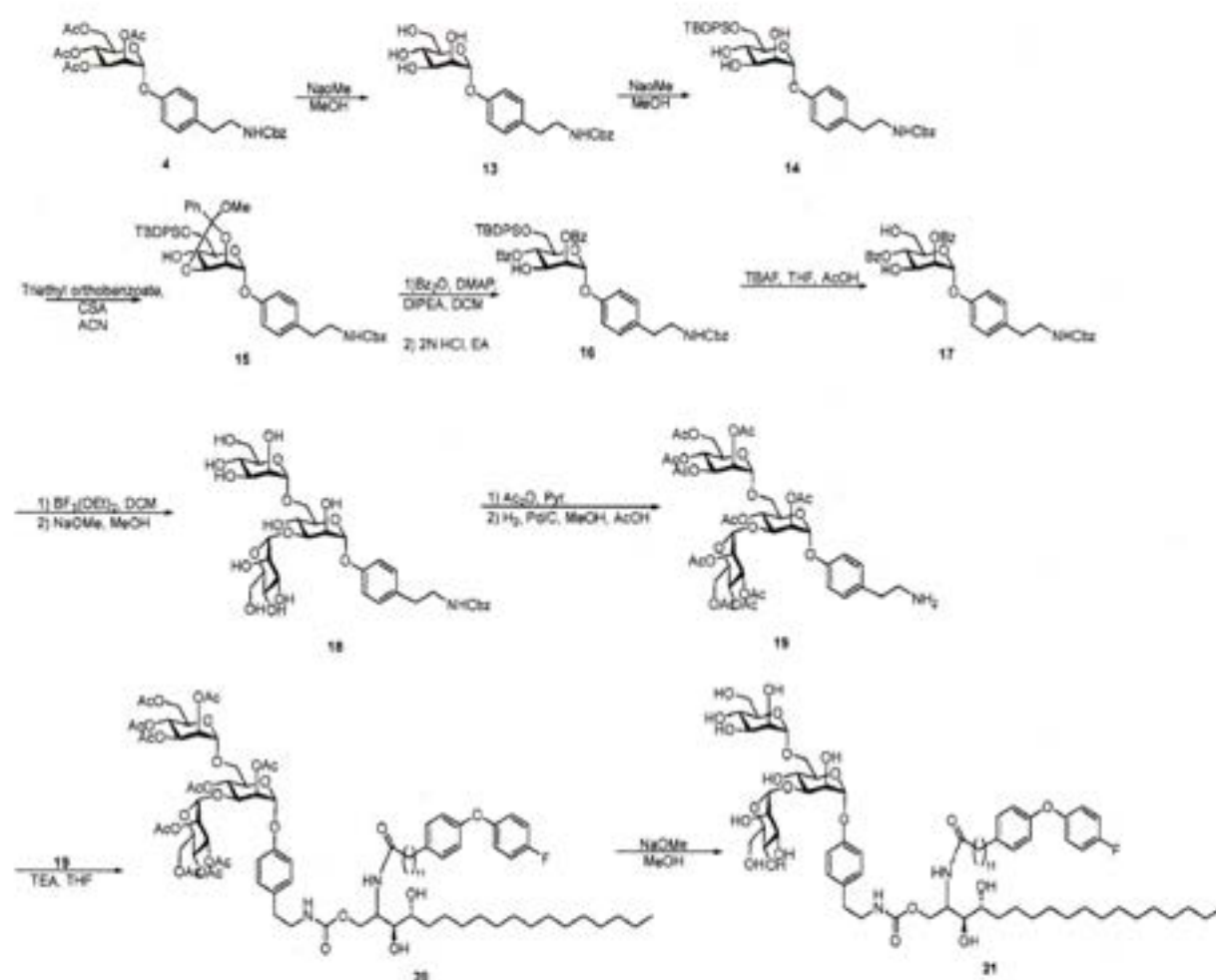
[0175] *Synthesis and Results*

[0176] 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.

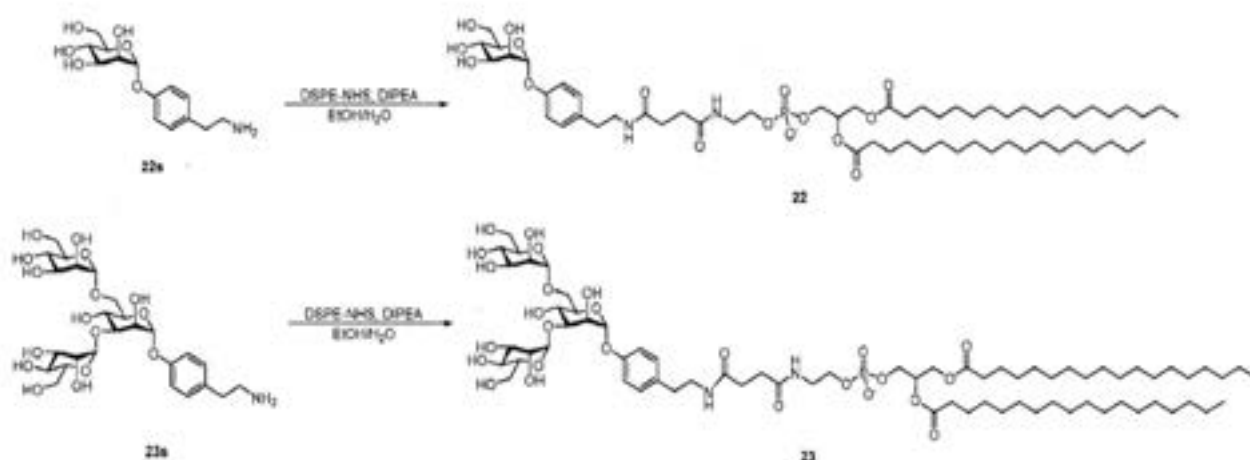
[0177] Scheme 1



[0178] Scheme 2



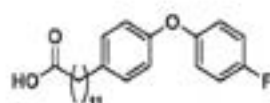
[0179] Scheme 3



[0180] Compounds 1 to 5

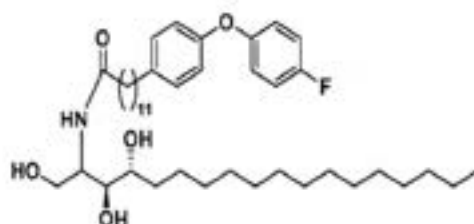
[0181] Compounds 1-5 were synthesized and characterized according to a published protocol (ACS Nano 2021, 15, 309–321).

[0182] Compound 8



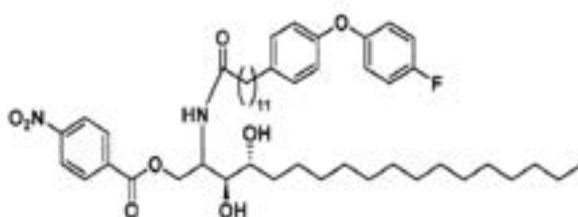
[0183] (11-Carboxynonyl)triphenylphosphonium bromide 6 (2.5 g, 10 mmol) was prepared by refluxing triphenylphosphine (10 mmol) and 11-bromoundecanoic acid (10 mmol). It was then dissolved in 50 ml of tetrahydrofuran (THF) and cooled to 0 °C. lithium bis(trimethylsilyl)amide (LHMDS; 1 M in THF, 20 mmol) 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 MgSO₄. 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 H₂ 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%).

[0184] Compound 9



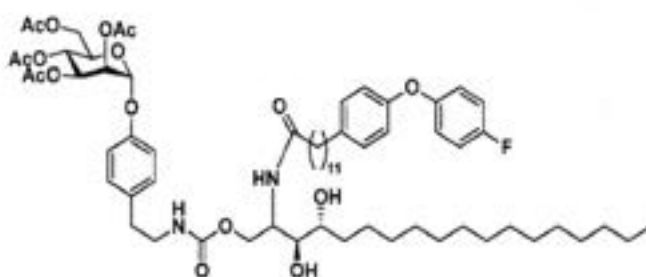
[0185] Compound 9. Compound 8 (1 mmol) in THF (10 mL) was added EDC (1.5 mmol), HOBt (1.5 mmol), DMAP (0.1 mmol), trimethylamine (2 mmol), and phytosphingosine (1.2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1) to yield 9 (74%).

[0186] **Compound 10**



[0187] 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.

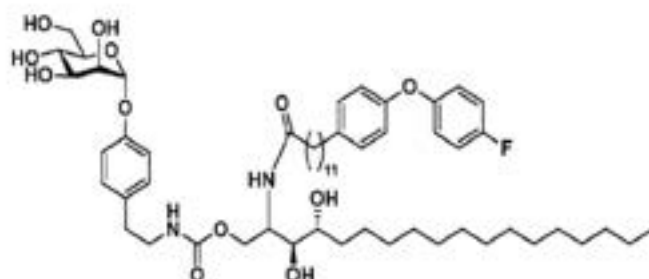
[0188] **Compound 11**



[0189] 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/H₂O. The collected organic layer was washed with

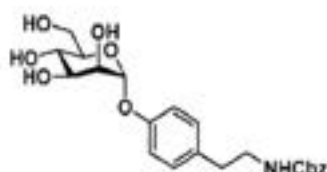
saturated $\text{NaHCO}_3(\text{aq})$, water and brine, and dried over MgSO_4 . The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **11** (59%).

[0190] **Compound 12**



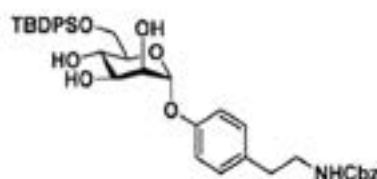
[0191] Compound **11** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at room temperature for 2 hours. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **12** (quant.).

[0192] **Compound 13**



[0193] 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.).

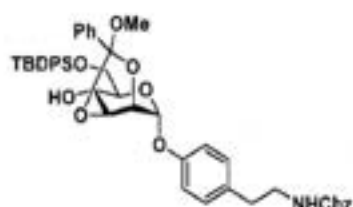
[0194] **Compound 14**



[0195] 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

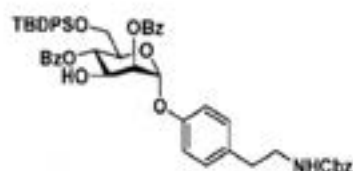
MeOH. After stirring at room temperature for 10 min, the solvent was removed under reduced pressure to give a dry residue that was purified by column chromatography with MeOH/DCM (1/10) to give compound **14** (82%).

[0196] Compound 15



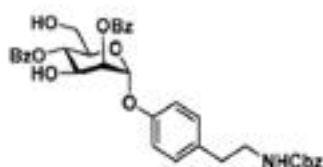
[0197] To a solution of compound **14** (1 mmol) and a catalytic amount of CSA (0.1 mmol) in CH₃CN (20 mL) was added trimethyl orthobenzoate (3 mmol) at room temperature under atmospheric pressure of nitrogen. After stirring for 30 min, Et₃N was added to quench the reaction, and the resulting mixture was dried under reduced pressure. The residue was purified by column chromatography with EA/Hex (1/2) to give compound **15** (79%).

[0198] **Compound 16**



[0199] 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/H₂O. The collected organic layer was washed with ice-cold saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **16** (71%).

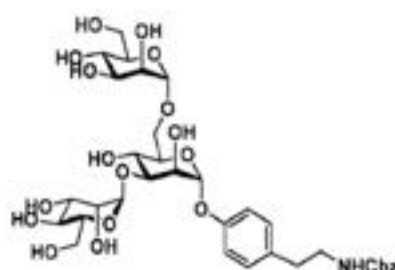
[0200] **Compound 17**



[0201] 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 NaHCO₃(aq), water, and brine, dried with anhydrous MgSO₄, and concentrated under reduced pressure. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound 47 (88%).

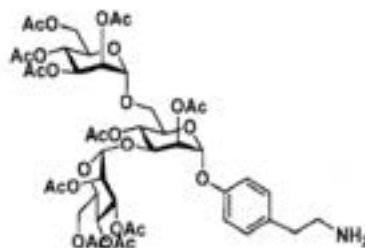
[0202]

[0203] **Compound 18**



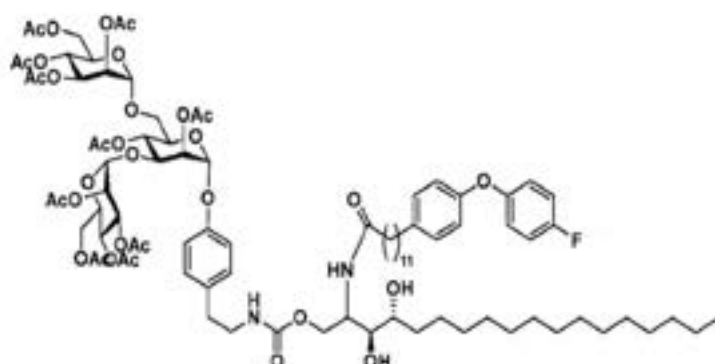
[0204] To a stirred solution of 17 (1 mmol) and 4 Å molecular sieve (0.1 g) in anhydrous DCM (10 mL) was cooled to -40°C and then BF₃(OEt)₂ (0.1 mmol) was added dropwise to the solution. A solution of 3 in anhydrous DCM was added dropwise to the above mixture and stirred for 1 h at -40°C. After that, 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 NaHCO₃ aq. and extracted with DCM. The organic layer was dried with MgSO₄ 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 H₂O as eluent to obtain a pure trisaccharide. The compound was lyophilized to dryness to give compound 18 (39%).

[0205] **Compound 19**



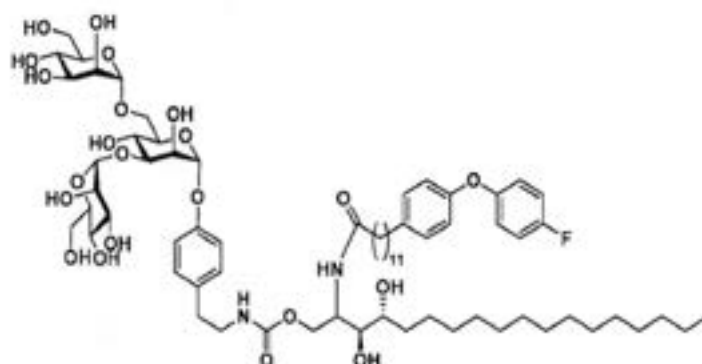
[0206] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo.

[0207] **Compound 20**

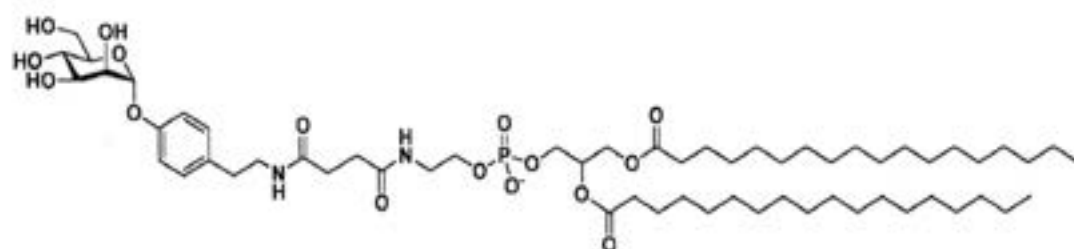


[0208] Compound **19** (1 mmol) in THF (10 mL) was added **10** (1 mmol) and trimethylamine (2 mmol), and the resulting solution was stirred under nitrogen at rt for 2 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **20**.

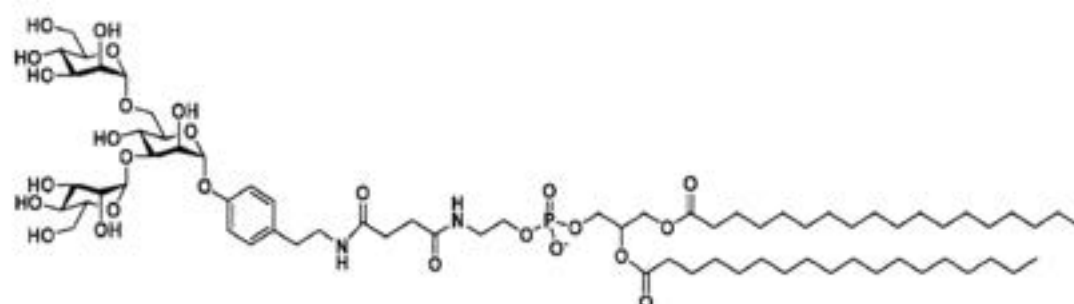
[0209] **Compound 21**



[0210] 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).

[0211] **Compound 22**

[0212] Arylmannoside **22s** (0.1 mmol) in EtOH/H₂O (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 H₂O as eluent to yield **22** (79%).

[0213] **Compound 23**

[0214] Aryltrimannoside **23s** (0.1 mmol) in EtOH/H₂O (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 H₂O as eluent to yield **23** (76%).

[0215] **Example 2: Preparation and characterization of the LNP of the present disclosure**[0216] *Preparation of LNP*

[0217] 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.

[0218] *Characterization of LNP*

[0219] *Size Measurement.* The LNP prepared above was examined using dynamic light scattering (DNP) to measure its size. First, 5 μ L of the LNP solution was transferred to a clean 1.5 mL tube and diluted with 95 μ L 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.

[0220] Table: the size and PDI measurement

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

[0221] *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 μ L) was prepared to obtain a standard curve. 5 μ L of the LNP solution prepared above was transferred to a clean tube and diluted to a final volume of 100 μ L. The working solution of the quant-it Ribogreen reagent (100 μ L) 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.

[0222] Table: Zeta potential and encapsulation efficiency

Sample	Zeta potential (mV)	Encapsulation efficiency (%)
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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

[0223] **Example 3: *In vitro* uptake and transfection of mRNA-LNPs in Dendritic cells.**

[0224] *Experiment 3-1*

[0225] *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 μ M 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 μ M 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.

[0226] 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

[0227] *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 °C 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).

[0228] *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).

[0229] *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-BMDCs, 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.

[0230] 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

[0231] *Experiment 3-2*

[0232] 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, 1x10⁶ 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.

[0233] 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°C for 48 hours. The experiments were conducted using FACS, similar to that described above.

[0234] 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

[0235] *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.

[0236] 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

[0237] *Experiment 3-3*

[0238] 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 CaCl₂, 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 tetramethylbenzidine (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 K_d was calculated using a nonlinear regression curve fit for total binding using GraphPad Prism.

[0239] **Example 4: *In vivo* delivery of luciferase mRNA-LNP**

[0240] 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 μ L) and maintained for one hour or six hours before *In vivo* Imaging System (IVIS®) measurement. For the IVIS measurement, the animals were first

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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 CO₂ 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.

[0241] 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

[0242] *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.

[0243] Additionally, exemplary LNPs, according to the present disclosure, are prepared to carry not only the mRNAs encoding luciferase but also either an mRNA encoding wide-type SARS-CoV-2 spike proteins or an mRNA encoding a low-sugar profile SARS-CoV-2 spike proteins. Mice are 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 are first anesthetized using the rodent anesthesia system with isoflurane (2.5% (vol/vol) in 0.2 L/min O₂ flow). Then, the animals are injected intravenously with D-luciferin solution (dissolved in 1X PBS; 150 mg/kg body weight). After 3 minutes from the injection, the animals are scanned using the IVIS imaging system (data not shown). After imaging, the animals are euthanized in a CO₂ chamber. The organs (heart, lungs, liver, spleen, kidneys, and lymph nodes) of the animals are collected, and the luminescence is detected and quantified using the IVIS system.

[0244] **Example 6: Immunization**

[0245] *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).

[0246] *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.

[0247] *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.

[0248] *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 titrating pseudotyped lentivirus,

different amounts of lentivirus were added into the culture medium containing polybrene (final concentration 8 $\mu\text{g/ml}$). Spin infection was carried out at 1,100 $\times g$ 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 $\mu\text{g/ml}$ 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 $(\text{RLU}^{\text{control}} - \text{RLU}^{\text{Serum}})/\text{RLU}^{\text{control}}$.

[0249] *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.

[0250] *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.

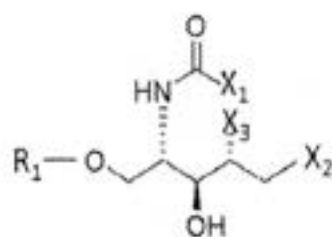
[0251] **FIG. 10** verifies that the LNPs of the present disclosure were capable of inducing antigen-specific IgG *in vivo*. The LNP, carrying mRNA encoding wide-type spike protein, induced IgGs that were still able to recognize the spike proteins of both the Delta variant and Omicron variant at a good 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.

[0252] 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.

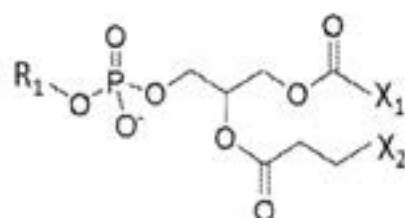
[0253] **FIG. 11** shows the results of an additional experiment. In this experiment, a Moderna LNP was prepared using Moderna's proprietary formulation. In addition, LNP made using the Moderna formulation and adding a compound of the present disclosure was also prepared to test whether the compound 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.

[0254] **EXEMPLARY EMBODIMENTS**

[0255] Embodiment A1. A pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle comprises 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 bi-functional compound comprising:



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; wherein the membrane encapsulates a payload encoding a spike protein of SARS-CoV-2 wherein the spike protein has a reduced and/or deleted glycan profile.

[0256] Embodiment A2. The pharmaceutical formulation of Embodiment 1, wherein the spike protein comprises an amino acid substitution of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue.

[0257] Embodiment A3. The pharmaceutical formulation of Embodiment A1 or Embodiment A2, wherein the spike protein comprises an amino acid deletion or addition at N-linked glycosylation sequons (N-X-S/T) to eliminate N-linked glycan sequons.

[0258] Embodiment A4. The pharmaceutical formulation of any one of Embodiments A1 to A3, wherein the spike protein comprises an amino acid substitution of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites.

[0259] Embodiment A5. The pharmaceutical formulation of any one of Embodiments A1 to A4, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 2, 16, 18, or 20, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to

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

[0260] Embodiment A6. The pharmaceutical formulation of any one of Embodiments A1 to A5, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, 22, 24, or 26, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 4, 22, 24, or 26, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation sites; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 3, 21, 23, or 25 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 3, 21, 23, or 25 respectively.

[0261] Embodiment A7. The pharmaceutical formulation of any one of Embodiments A1 to A6, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 6, 28, 30, or 32, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 6, 28, 30, or 32, and wherein the spike protein comprises a S2 subunit lacking at least one glycosylation sites; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 5, 27, 29, or 31 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 5, 27, 29, or 31 respectively.

[0262] Embodiment A8. The pharmaceutical formulation of any one of Embodiments A1 to A7, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 8 or 34, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 8 or 34, and wherein the spike protein comprises an S2 subunit comprising a single glycosylation site at N1194; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 7 or 33 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 7 or 33 respectively.

[0263] Embodiment A9. The pharmaceutical formulation of any one of Embodiments A1 to A8, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 10 or 36, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the

amino acid sequence of SEQ ID NO: 10 or 36, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation sites and an amino acid substitution of N801Q; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 9 or 35 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 9 or 35 respectively.

[0264] Embodiment A10. The pharmaceutical formulation of any one of Embodiments A1 to A9, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 12 or 38, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 12 or 38, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation sites and an amino acid substitution of N1194Q; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 11 or 37 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 11 or 37 respectively.

[0265] Embodiment A11. The pharmaceutical formulation of any one of Embodiments A1 to A10, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 14 or 40, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 14 or 40, and wherein the spike protein comprises a receptor binding domain (RBD) lacking at least one glycosylation sites and an amino acid substitution of N122Q, N165Q, N234Q, or a combination thereof; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 13 or 39 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set forth in SEQ ID NO: 13 or 39 respectively.

[0266] Embodiment A12. The pharmaceutical formulation of any one of Embodiments A1 to A11, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFRDIST (SEQ ID NO: 45), KPFRDISTEIYQAG (SEQ ID NO: 46), GPKKSTNLVKNNK (SEQ ID NO: 47), NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.

[0267] Embodiment A13. The pharmaceutical formulation of any one of Embodiments A1 to A11, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 53, 54, 55, 56, or 57, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 53, 54, 55, 56, or 57.

[0268] Embodiment A14. The pharmaceutical formulation of Embodiment A13, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group consisting of:

SEQ ID NO: 58 - SSANNCTFEYVSQ;
 SEQ ID NO: 59 - TESIVRFPNITNL;
 SEQ ID NO: 60 - KPFERDISTEIYQAG;
 SEQ ID NO: 61 - GPKKSTNLVKNC;
 SEQ ID NO: 62 - TEVPVAIHADQ;
 SEQ ID NO: 63 - RVYSTGSNVFQTR;
 SEQ ID NO: 64 - RRARSVASQS;
 SEQ ID NO: 65 - DPSKPSKRSF;
 SEQ ID NO: 66 - FIKQYGDCLGDI;
 SEQ ID NO: 67 - ENQKLIANQFNS;
 SEQ ID NO: 68 - GKIQDLSSTA;
 SEQ ID NO: 69 - NCDVVIGIVNNTVY;
 SEQ ID NO: 70 - PELDSFKEELDKYFKNHTS;
 SEQ ID NO: 71 - TSPDVLGDISGINA;
 SEQ ID NO: 72 - VNIQKEIDRLNEVA;
 SEQ ID NO: 73 - NLNESLIDLQ; and
 SEQ ID NO: 74 - LGKYEQYIKWP;

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

[0269] Embodiment A15. The pharmaceutical formulation of any one of Embodiments A1 to A14, wherein R_1 comprises a formula of R_2-R_A , wherein R_2 is the substituted or non-substituted glycosyl group, 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.

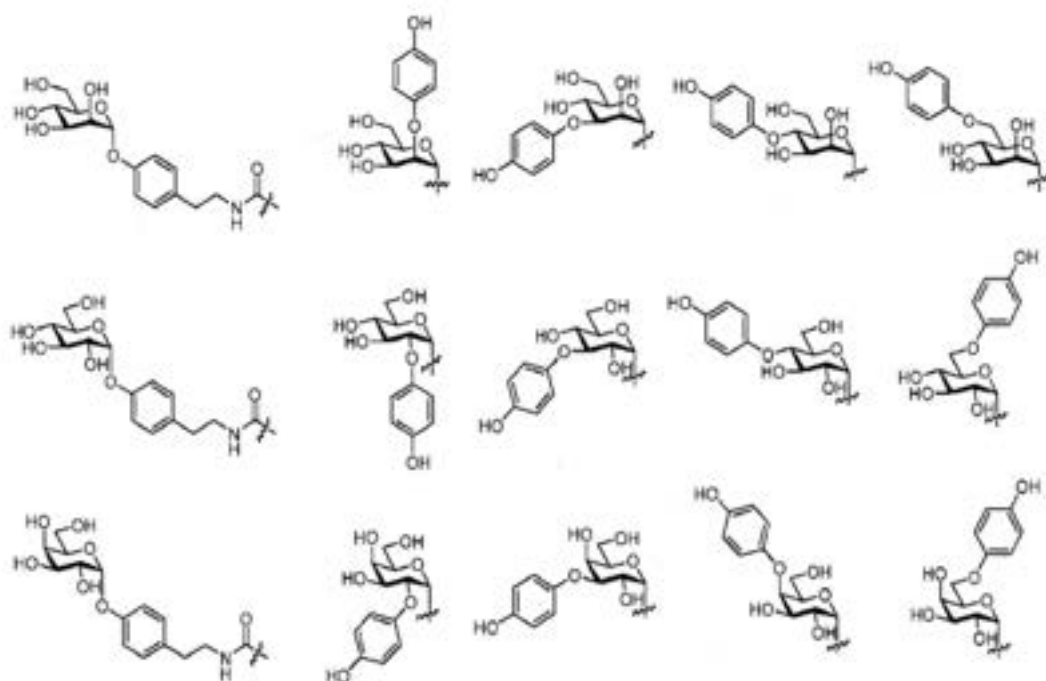
[0270] Embodiment A16. The pharmaceutical formulation of Embodiment A15, wherein R_A comprises the aryl having 0 to 3 substituents (e.g., 1 to 3 substituents), wherein the substituent is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide.

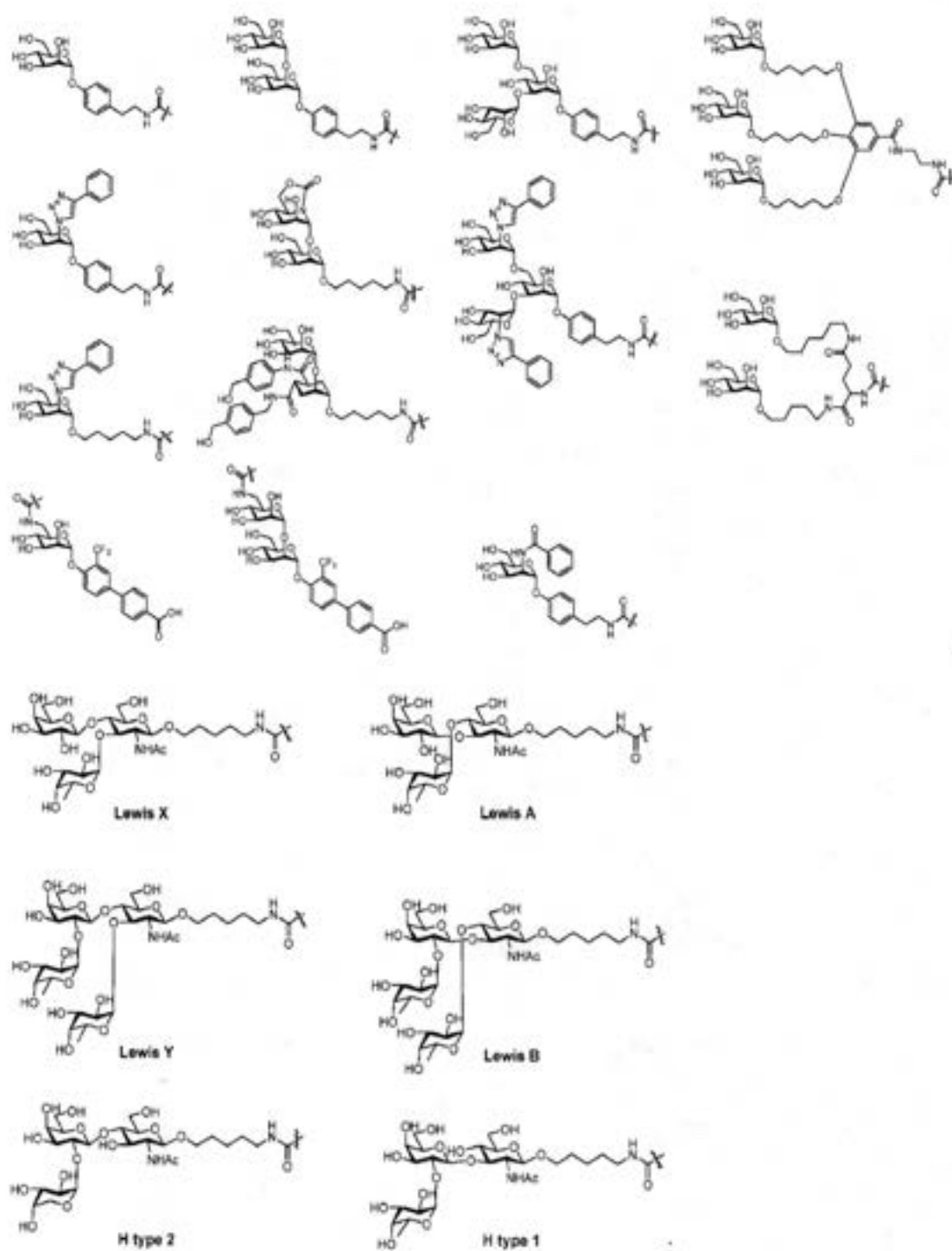
[0271] Embodiment A17. The pharmaceutical formulation of Embodiment A16, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.

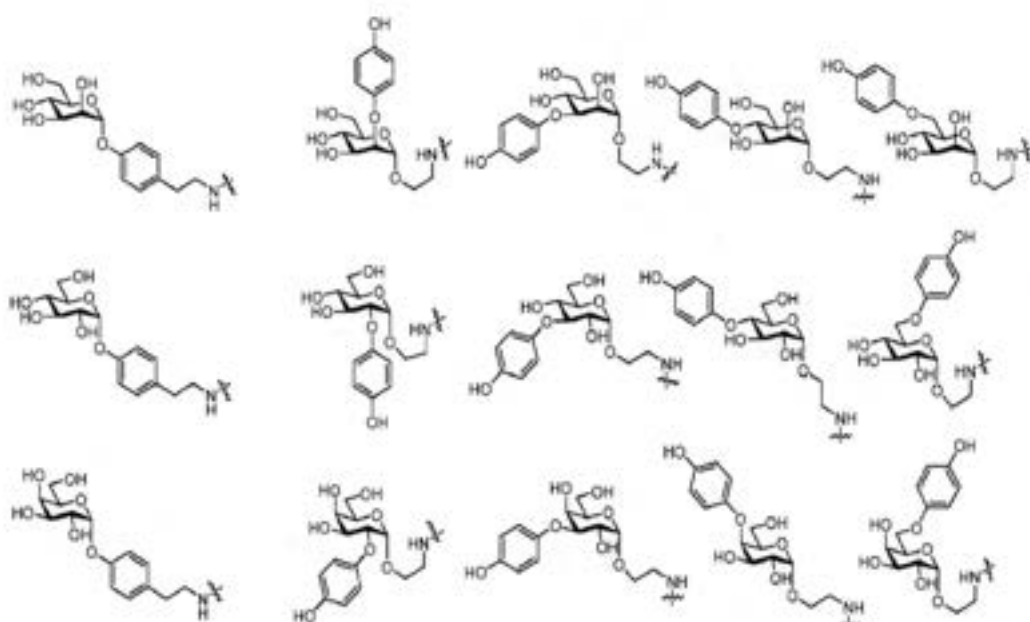
- [0272] Embodiment A18. The pharmaceutical formulation of Embodiment A16 or Embodiment A17, wherein the PEG moiety is linear.
- [0273] Embodiment A19. The pharmaceutical formulation of any one of Embodiments A1 to A18, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.
- [0274] Embodiment A20. The pharmaceutical formulation of any one of Embodiments A1 to A19, wherein the glycosyl group comprises a terminal mannoside, a terminal fucoside, or both.
- [0275] Embodiment A21. The pharmaceutical formulation of any one of Embodiments A1 to A20, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
- [0276] Embodiment A22. The pharmaceutical formulation of Embodiment A21, wherein the tri-mannoside is a linear or branched tri-mannoside.
- [0277] Embodiment A23. The pharmaceutical formulation of Embodiment A22, wherein the branched tri-mannoside is a α -1,3- α -1,6-trimannoside.
- [0278] Embodiment A24. The pharmaceutical formulation of any one of Embodiments A1 to A23, wherein R_1 is a substituted glycosyl group.
- [0279] Embodiment A25. The pharmaceutical formulation of Embodiment A24, 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.
- [0280] Embodiment A26. The pharmaceutical formulation of Embodiment A25, 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.
- [0281] Embodiment A27. The pharmaceutical formulation of Embodiment A25 or Embodiment A26, 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.

[0282] Embodiment A28. The pharmaceutical formulation of Embodiment A25 or Embodiment A26, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.

[0283] Embodiment A29. The pharmaceutical formulation of any one of Embodiments A1 to A28, wherein R₁ is selected from the group consisting of:



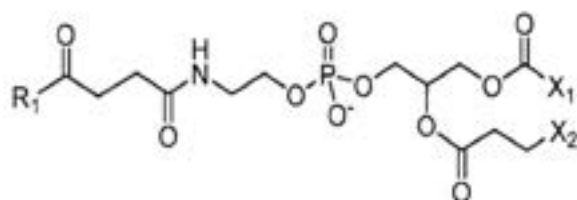




[0284] Embodiment A30. The pharmaceutical formulation of any one of Embodiments A1 to A29, wherein the compound is of Formula 1.

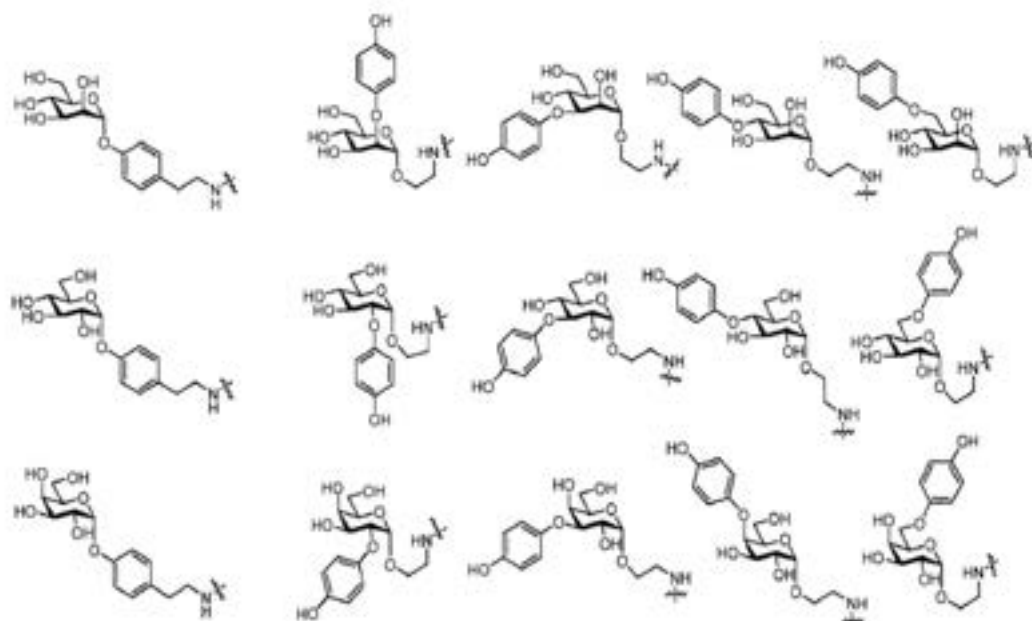
[0285] Embodiment A31. The pharmaceutical formulation of any one of Embodiments A1 to A30, wherein the compound is of Formula 2.

[0286] Embodiment A32. The pharmaceutical formulation of Embodiment A31, wherein the compound is of Formula 3:



Formula 3; and

wherein R_1 is selected from the group consisting of:



[0287] Embodiment A33. The pharmaceutical formulation of any one of Embodiments A1 to A32, 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.

[0288] Embodiment A34. The pharmaceutical formulation of any one of Embodiments A1 to A33, 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.

[0289] Embodiment A35. The pharmaceutical formulation of Embodiment A34, wherein 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.

[0290] Embodiment A36. The pharmaceutical formulation of any one of Embodiments A1 to A35, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen.

[0291] Embodiment A37. The pharmaceutical formulation of any one of Embodiments A1 to A36, 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.

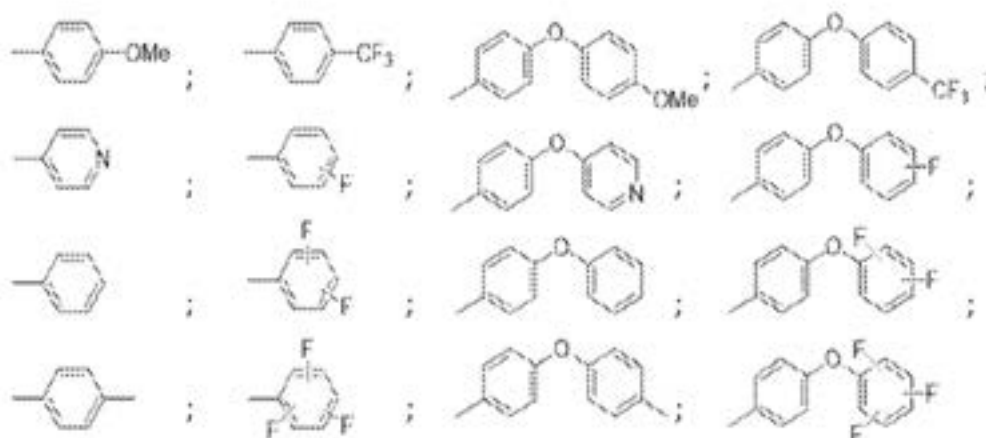
[0292] Embodiment A38. The pharmaceutical formulation of Embodiment A37, wherein the substituent is CH_3 , CF_3 , F, or OCH_3 .

[0293] Embodiment A39. The pharmaceutical formulation of Embodiment A37 or Embodiment A38, wherein X_4 comprises 1 to 3 substituents.

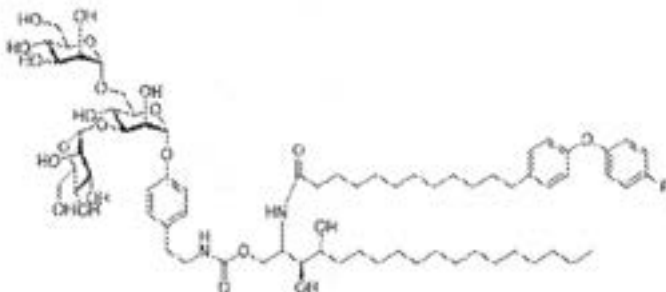
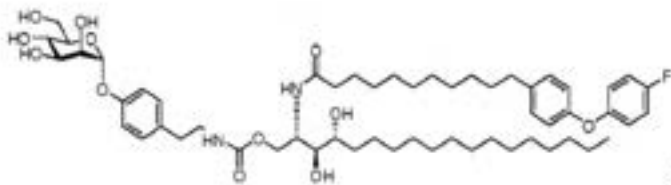
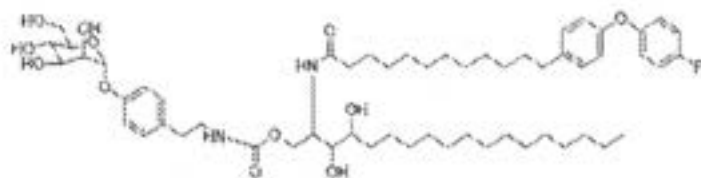
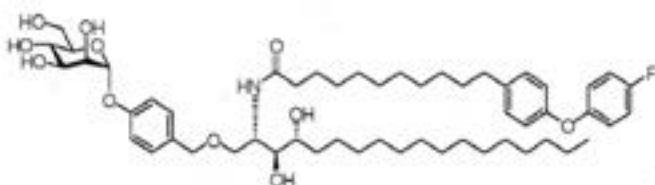
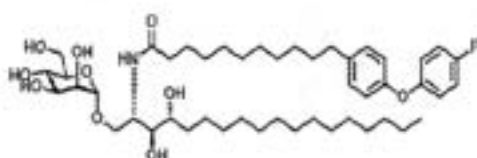
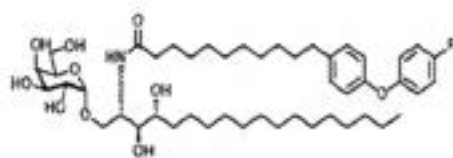
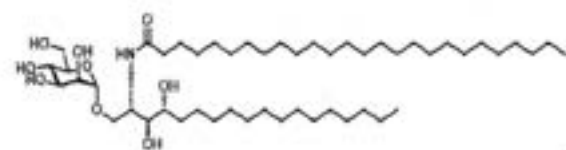
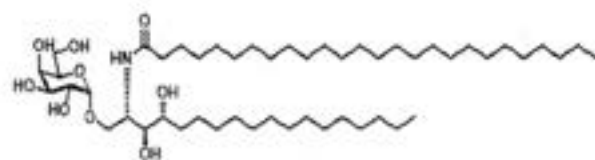
[0294] Embodiment A40. The pharmaceutical formulation of any one of Embodiments A37 to A39, wherein X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

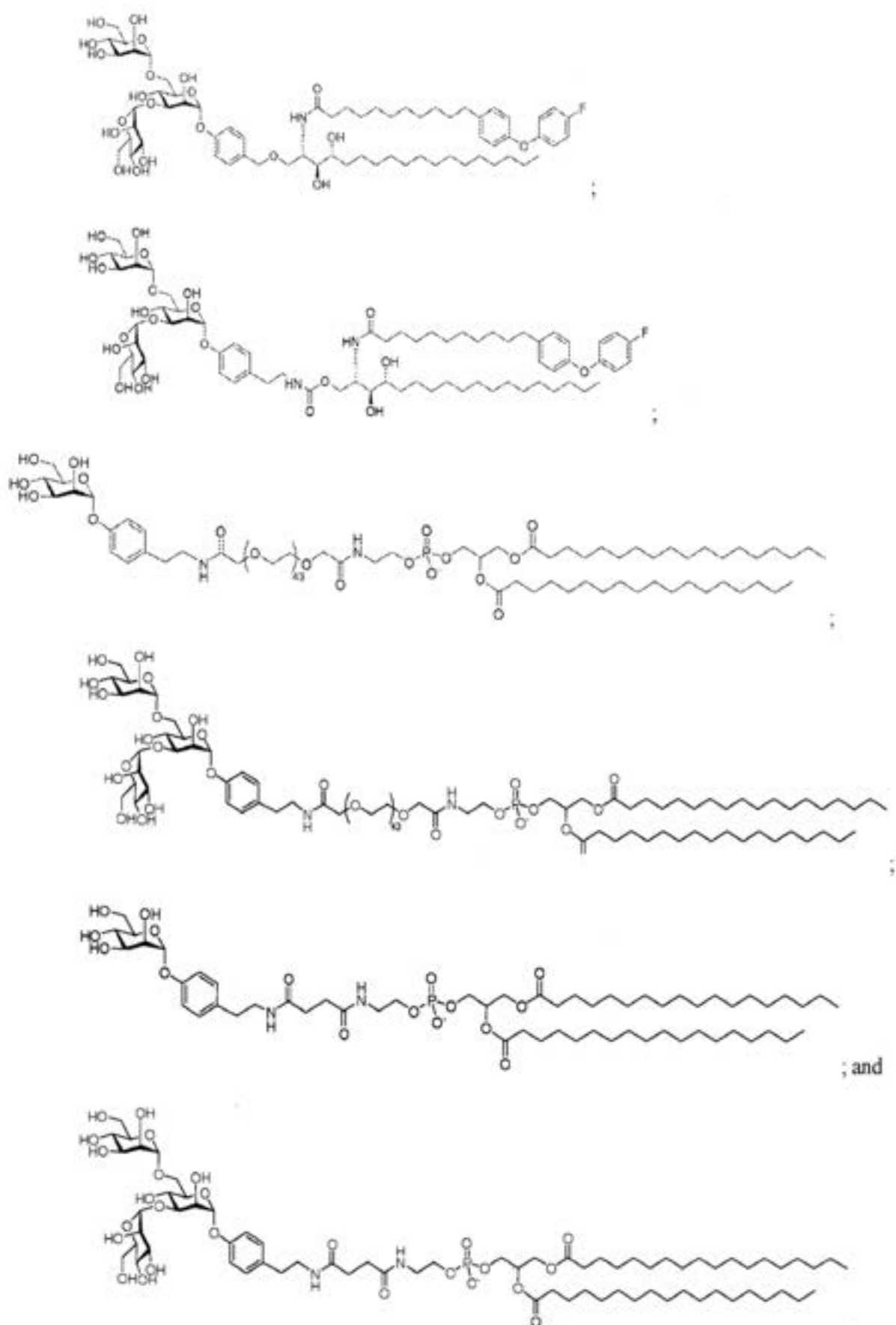
[0295] Embodiment A41. The pharmaceutical formulation of any one of Embodiments A1 to A40, wherein one of X_1 and X_2 is C_{15-30} alkyl, and the other one is $-(CH_2)_nX_4$.

[0296] Embodiment A42. The pharmaceutical formulation of any one of Embodiments A1 to A41, wherein X_4 is selected from the group consisting of:



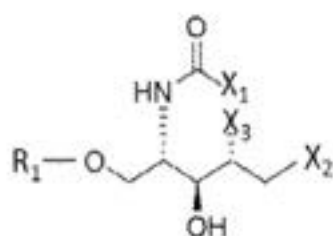
[0297] Embodiment A43. The pharmaceutical formulation of any one of Embodiments A1 to A42, wherein the compound is selected from the group consisting of:



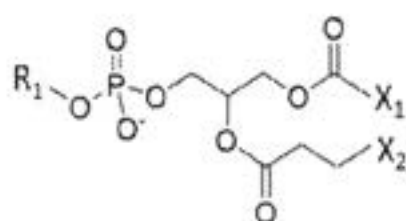


- [0298] Embodiment A44. The pharmaceutical formulation of any one of Embodiments A1 to A43, wherein the component is not glycolipid C34 or α -galactosylceramide.
- [0299] Embodiment A45. The pharmaceutical formulation of any one of Embodiments A1 to A44, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.
- [0300] Embodiment A46. The pharmaceutical formulation of any one of Embodiments A1 to A45, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.
- [0301] Embodiment A47. The pharmaceutical formulation of Embodiment A46, wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.
- [0302] Embodiment A48. The pharmaceutical formulation of any one of Embodiments A1 to A47, 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.
- [0303] Embodiment A49. The pharmaceutical formulation of any one of Embodiments A1 to A48, further comprising an adjuvant.
- [0304] Embodiment A50. The pharmaceutical formulation of Embodiment A49, 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.
- [0305] Embodiment A51. A method of targeted payload delivery in a subject, comprising administering to the subject an effective amount of the pharmaceutical formulation of any one of Embodiments A1 to A50.
- [0306] Embodiment A52. The method of Embodiment A51, wherein the pharmaceutical formulation is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.

- [0307] Embodiment A53. The method of Embodiment A51 or Embodiment A52, 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.
- [0308] Embodiment A54. The method of any one of Embodiments A51 to A53, wherein the effective amount ranges from about 5 μg to 1000 μg .
- [0309] Embodiment A55. A method of preventing or treating a disease in a subject, comprising administering to the subject an effective amount of the pharmaceutical formulation of any one of Embodiments A1 to A50.
- [0310] Embodiment A56. The method of Embodiment A55, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.
- [0311] Embodiment A57. The method of Embodiment A55 or Embodiment A56, 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.
- [0312] Embodiment A58. The method of any one of Embodiments A55 to A57, wherein the effective amount ranges from about 5 μg to 1000 μg .
- [0313] Embodiment A59. A method of boosting an adaptive immune response, comprising administering to the subject an effective amount of the pharmaceutical formulation of any one of Embodiments A1 to A50.
- [0314] Embodiment A60. The method of Embodiment A59, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.
- [0315] Embodiment A61. The method of Embodiment A59 or Embodiment A60, 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.
- [0316] Embodiment A62. The method of any one of Embodiments A59 to A61, wherein the effective amount ranges from about 5 μg to 1000 μg .
- [0317] Embodiment B1. 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.

[0318] Embodiment B2. The compound of embodiment B1, 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.

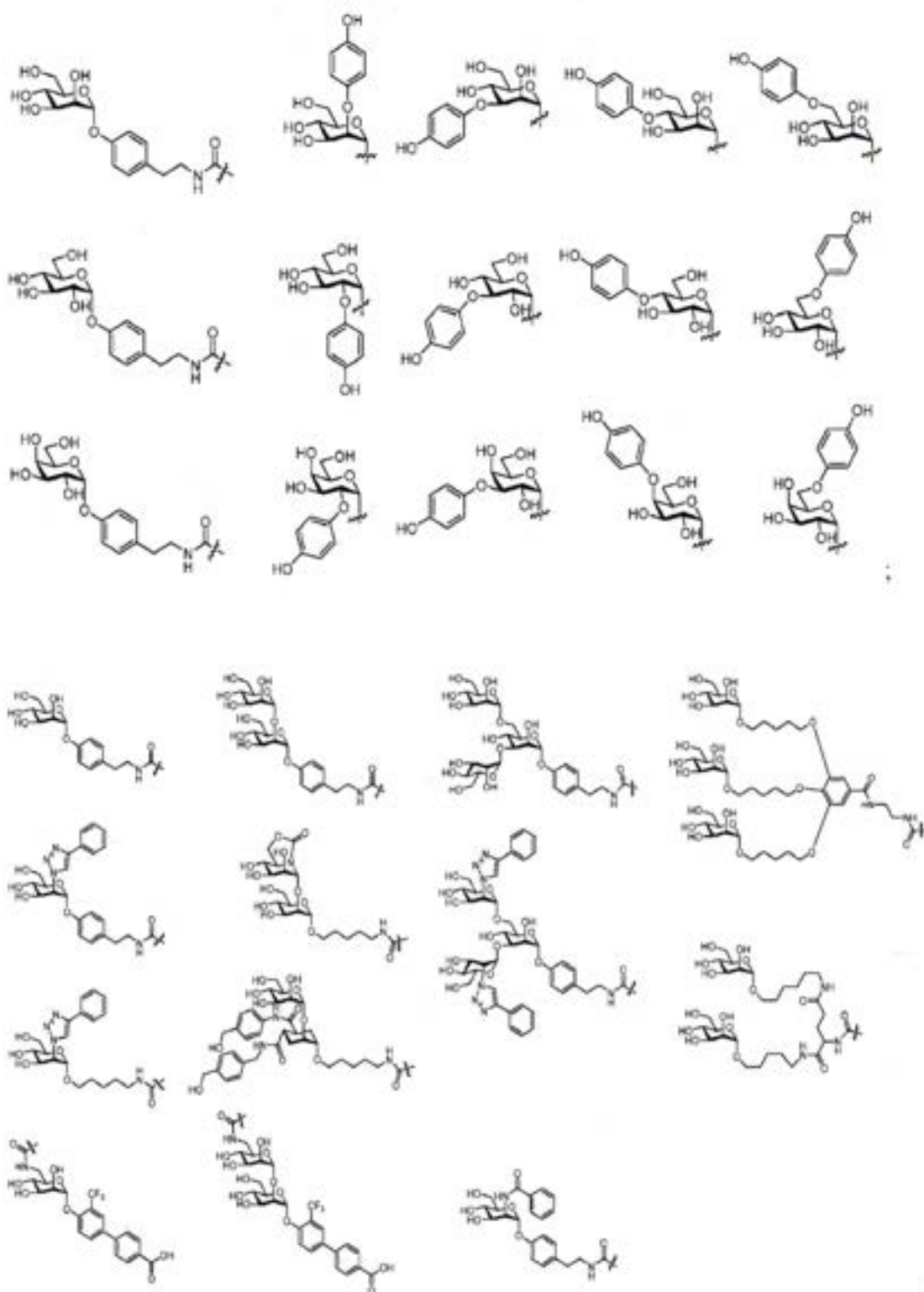
[0319] Embodiment B3. The compound of embodiment B2, wherein R_A comprises the aryl having 0 to 3 substituents, wherein the substituent is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide.

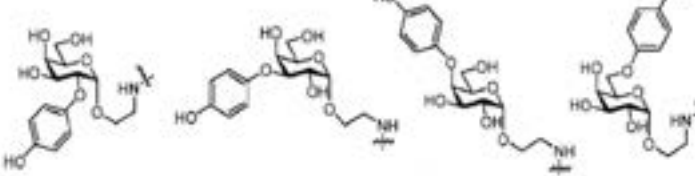
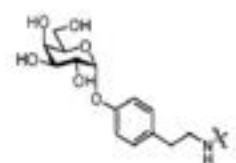
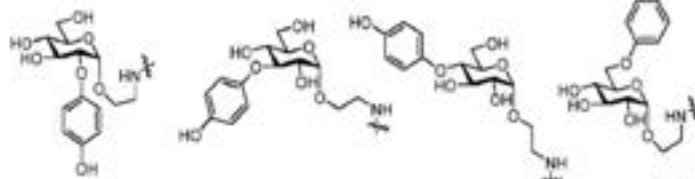
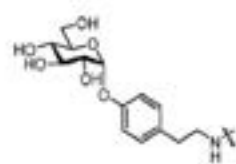
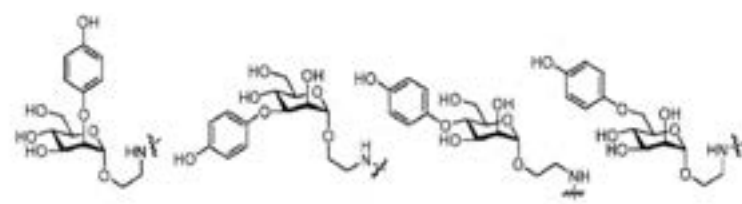
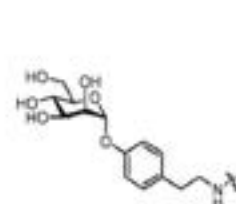
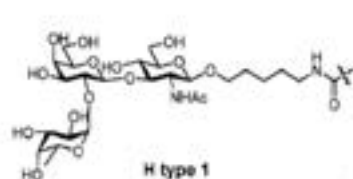
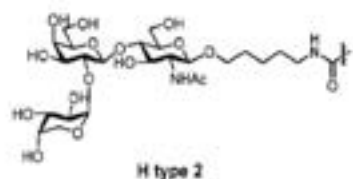
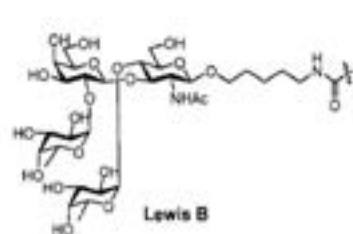
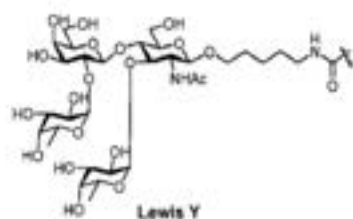
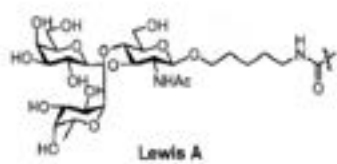
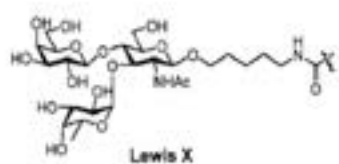
[0320] Embodiment B4. The compound of embodiment B3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.

[0321] Embodiment B5. The copolymer of embodiment B3 or embodiment B4, wherein the PEG moiety is linear.

[0322] Embodiment B6. The compound of any one of embodiments B1 to B5, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.

- [0323] Embodiment B7. The compound of any one of embodiments B1 to B6, wherein the glycosyl group comprises a terminal mannoside, a terminal fucoside, or both.
- [0324] Embodiment B8. The compound of any one of embodiments B1 to B7, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
- [0325] Embodiment B9. The compound of embodiment B8, wherein the tri-mannoside is a linear or branched tri-mannoside.
- [0326] Embodiment B10. The compound of embodiment B9, wherein the branched tri-mannoside is a α -1,3- α -1,6-trimannoside.
- [0327] Embodiment B11. The compound of any one of embodiments B1 to B10, wherein R_1 is a substituted glycosyl group.
- [0328] Embodiment B12. The compound of embodiment B11, 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.
- [0329] Embodiment B13. The compound of embodiment B12, 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.
- [0330] Embodiment B14. The compound of embodiment B12 or embodiment B13, 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof.
- [0331] Embodiment B15. The compound of embodiment B12 or embodiment B13, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.
- [0332] Embodiment B16. The compound of any one of embodiments B1 to B15, wherein R_1 is selected from the group consisting of:

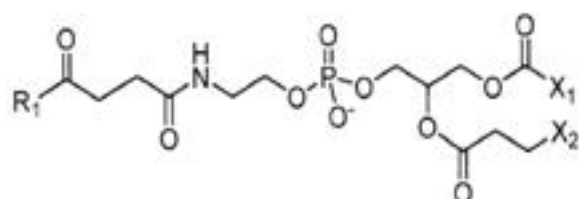




[0333] Embodiment B17. The compound of any one of embodiments B1 to B16, wherein the compound is of Formula 1.

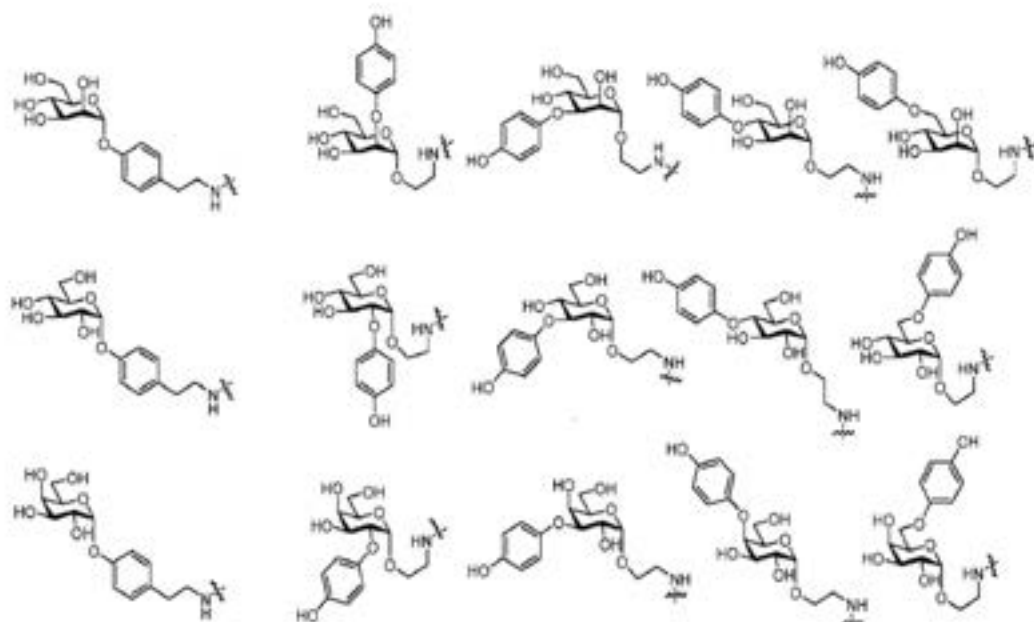
[0334] Embodiment B18. The compound of any one of embodiments B1 to B16, wherein the compound is of Formula 2.

[0335] Embodiment B19. The compound of embodiment B18, wherein the compound is of Formula 3:



Formula 3: and

wherein R₁ is selected from the group consisting of:



[0336] Embodiment B20. The compound of any one of embodiments B1 to B19, wherein at least one of X₁ and X₂ 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.

[0337] Embodiment B21. The compound of any one of embodiments B1 to B20, 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.

[0338] Embodiment B22. The compound of embodiment B21, wherein 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.

[0339] Embodiment B23. The compound of any one of embodiments B1 to B22, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen.

[0340] Embodiment B24. The compound of any one of embodiments B1 to B23, 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.

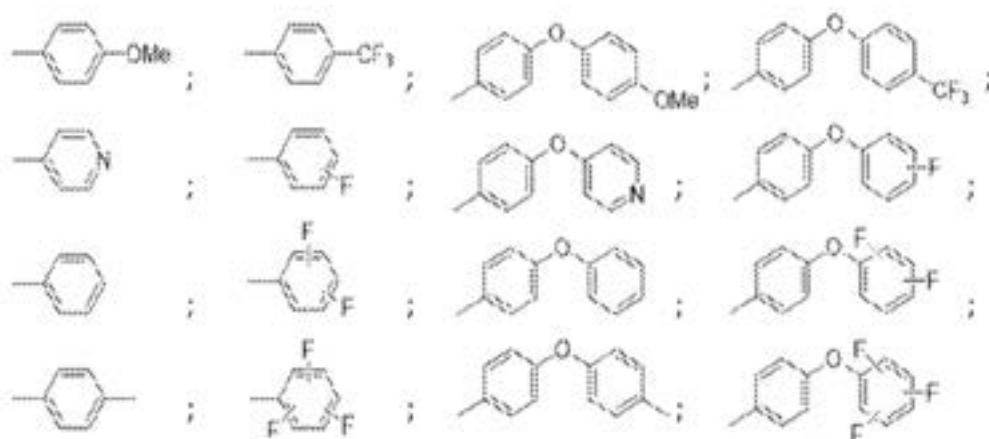
[0341] Embodiment B25. The compound of embodiment B24, wherein the substituent is CH_3 , CF_3 , F, or OCH_3 .

[0342] Embodiment B26. The compound of embodiment B24 or embodiment B25, wherein X_4 comprises 1 to 3 substituents.

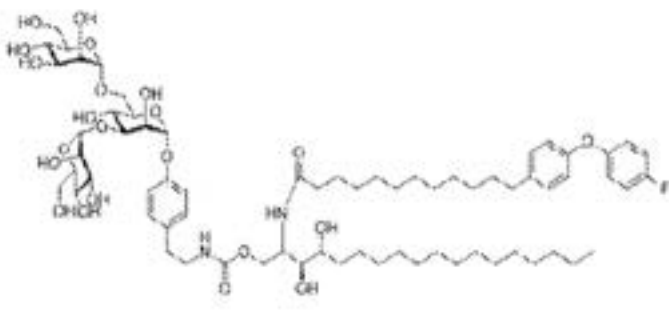
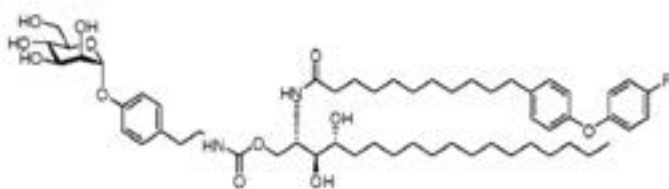
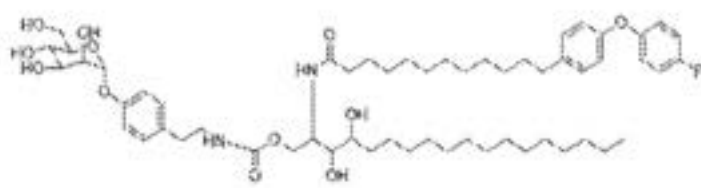
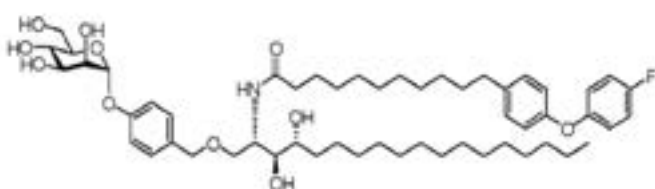
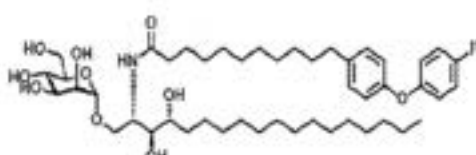
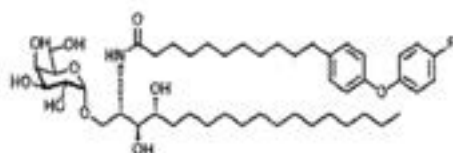
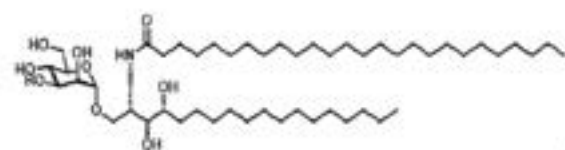
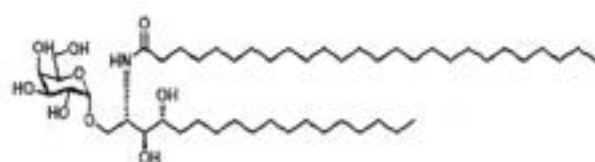
[0343] Embodiment B27. The compound of any one of embodiments B24 to B26, wherein X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

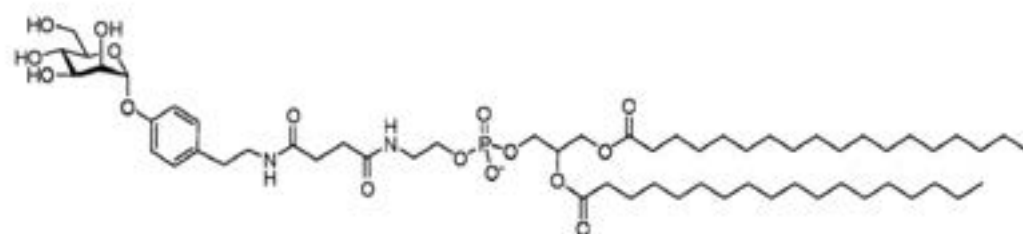
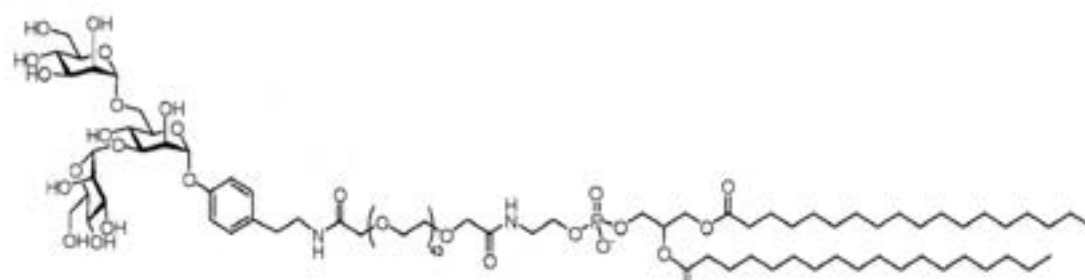
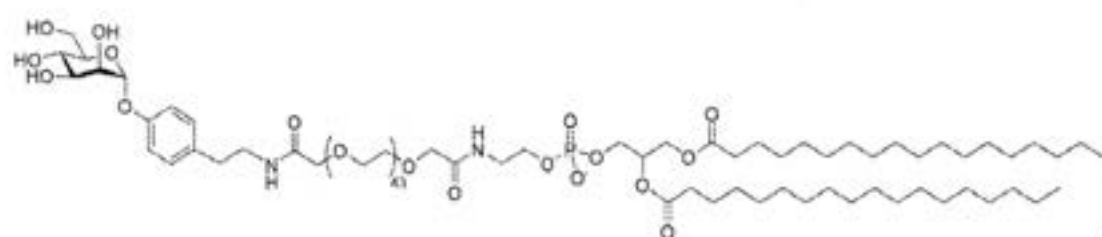
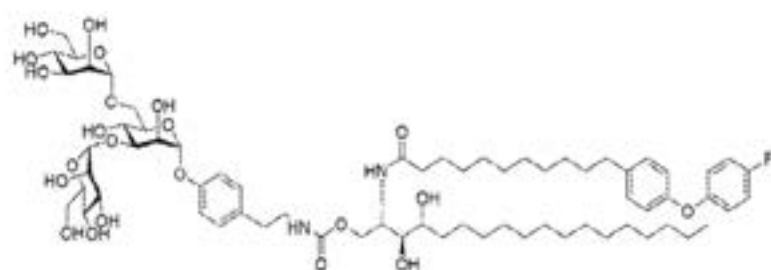
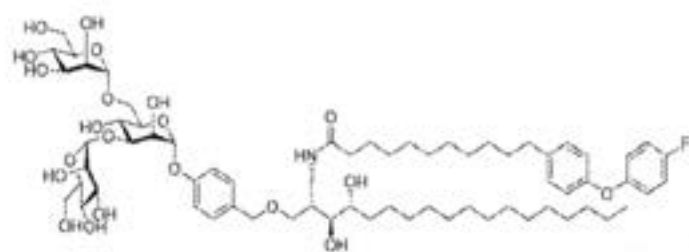
[0344] Embodiment B28. The compound of any one of embodiments B1 to B27, wherein one of X_1 and X_2 is C_{15-30} alkyl, and the other one is $-(CH_2)_nX_4$.

[0345] Embodiment B29. The compound of any one of embodiments B1 to B28, wherein X_4 is selected from the group consisting of:

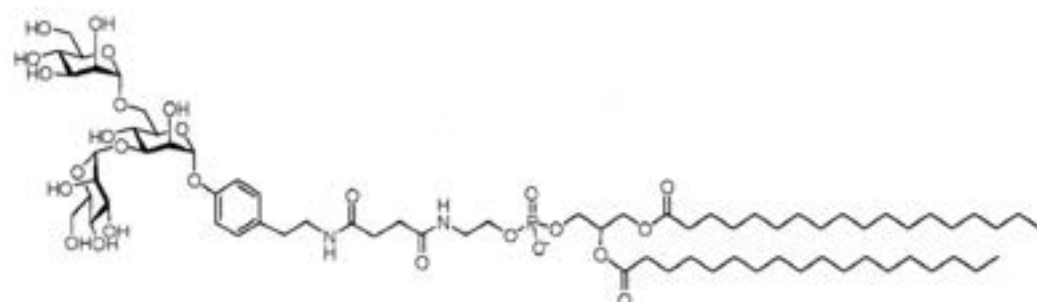


[0346] Embodiment B30. The compound of any one of embodiments B1 to B29, selected from the group consisting of:





; and



[0347] Embodiment B31. The component of any one of embodiments B1 to B30, wherein the component is not glycolipid C34 or α -galactosylceramide.

[0348] Embodiment B32. A formulation for forming a lipid nanoparticle, comprising the compound of any one of embodiments B1 to B31, wherein the compound comprises 1 to 10 mol% of the composition.

[0349] Embodiment B33. The formulation of embodiment B32, 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.

[0350] Embodiment B34. The formulation of embodiment B33, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0351] Embodiment B35. The formulation of embodiment B33 or embodiment B34, 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.

[0352] Embodiment B36. The formulation of embodiment B35, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0353] Embodiment B37. The formulation of embodiment B35 or embodiment B36, 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, (3 β ,5 β)-3-hydroxycholestan-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.

[0354] Embodiment B38. The formulation of any one of embodiments B35 to B37, 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.

[0355] Embodiment B39. The formulation of any one of embodiments B33 to B38, further comprising a payload.

[0356] Embodiment B40. The formulation of embodiment B39, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0357] Embodiment B41. The formulation of embodiment B40, wherein the nucleic acid is RNA or DNA.

[0358] Embodiment B42. The formulation of embodiment B41, wherein the payload encodes a polypeptide.

[0359] Embodiment B43. The formulation of any one of embodiments B39 to B42, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0360] Embodiment B44. The formulation of any one of embodiments B39 to B43, 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.

[0361] Embodiment B45. The formulation of embodiment B44, wherein the first payload and the second payload are different.

[0362] Embodiment B46. The formulation of any one of embodiments B32 to B45, further comprising a pharmaceutically acceptable excipient, adjuvant, or a combination thereof.

[0363] Embodiment B47. 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 B1 to B31.

[0364] Embodiment B48. The lipid nanoparticle of embodiment B47, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof.

[0365] Embodiment B49. The lipid nanoparticle of embodiment B47 or embodiment B48, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.

[0366] Embodiment B50. The lipid nanoparticle of embodiment B49, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0367] Embodiment B51. The lipid nanoparticle of embodiment B49 or embodiment B50, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

[0368] Embodiment B52. The lipid nanoparticle of embodiment B51, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0369] Embodiment B53. The lipid nanoparticle of embodiment B51 or embodiment B52, 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, (3 β ,5 β)-3-hydroxycholestan-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.

[0370] Embodiment B54. The lipid nanoparticle of any one of embodiments B51 to B53, 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.

[0371] Embodiment B55. The lipid nanoparticle of any one of embodiments B47 to B54, wherein the membrane encapsulates a payload.

[0372] Embodiment B56. The lipid nanoparticle of embodiment B55, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0373] Embodiment B57. The lipid nanoparticle of embodiment B56, wherein the nucleic acid is RNA or DNA.

[0374] Embodiment B58. The lipid nanoparticle of embodiment B57, wherein the payload encodes a polypeptide.

[0375] Embodiment B59. The lipid nanoparticle of any one of embodiments B55 to B58, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0376] Embodiment B60. The lipid nanoparticle of any one of embodiments B55 to B59, wherein the payload is a first payload, and the membrane further encapsulates a second payload.

[0377] Embodiment B61. The lipid nanoparticle of embodiment B60, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.

[0378] Embodiment B62. The lipid nanoparticle of embodiment B60 or embodiment B61, wherein the first payload and the second payload are different.

[0379] Embodiment B63. The lipid nanoparticle of any one of embodiments B47 to B62, being made from the composition of any one of embodiments B29 to B43.

[0380] Embodiment B64. The lipid nanoparticle of any one of embodiments B47 to B63, wherein the membrane is a bi-layer structure.

[0381] Embodiment B65. The lipid nanoparticle of any one of embodiments B47 to B64, having a diameter of 0.01 to 5 microns.

[0382] Embodiment B66. The lipid nanoparticle of any one of embodiments B47 to B65, wherein the plurality of the lipid components does not comprise glycolipid C34 or α -galactosylceramide (α -GalCer).

[0383] Embodiment B67. A formulation, comprising the lipid nanoparticle of any one of embodiments B47 to B66.

[0384] Embodiment B68. The formulation of embodiment B67, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.

[0385] 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.

[0386] Embodiment B70. The formulation of embodiment B69, wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.

[0387] Embodiment B71. The formulation of any one of embodiments B67 to B70, further comprising an excipient, an adjuvant, or a combination thereof.

[0388] Embodiment B72. The formulation of embodiment B71, 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.

[0389] Embodiment B73. The formulation of embodiment B71 or embodiment B72, 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.

[0390] Embodiment B74. A kit for preparing a lipid nanoparticle, comprising: a first reagent, comprising the compound of any one of embodiments B1 to B31; and a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.

[0391] Embodiment B75. The kit of embodiment B74, wherein the second reagent comprises the ionizable lipid.

[0392] Embodiment B76. The kit of embodiment B75, wherein the kit further comprises a third reagent comprising a helper lipid.

[0393] Embodiment B77. The kit of any one of embodiments B74 to B76, wherein ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediyldis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0394] Embodiment B78. The kit of any one of embodiments B74 to B77, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.

[0395] Embodiment B79. The kit of embodiment B78, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

[0396] Embodiment B80. The kit of embodiment B78 or embodiment B79, 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, (3 β ,5 β)-3-hydroxycholesterol-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.

[0397] Embodiment B81. The kit of any one of embodiments B78 to B80, 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.

[0398] Embodiment B82. The kit of any one of embodiments B74 to B81, 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.

[0399] Embodiment B83. The kit of embodiment B82, wherein the nucleic acid is RNA or DNA.

[0400] Embodiment B84. The kit of embodiment B83, wherein the payload encodes a polypeptide.

[0401] Embodiment B85. The kit of any one of embodiments B82 to B84, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.

[0402] Embodiment B86. 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.

[0403] Embodiment B87. The method of embodiment B86, wherein the nucleic acid is RNA or DNA.

- [0404] Embodiment B88. The method of embodiment B87, wherein the payload encodes a polypeptide.
- [0405] Embodiment B89. The method of any one of embodiments B86 to B88, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
- [0406] Embodiment B90. The method of any one of embodiments B86 to B89, 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.
- [0407] Embodiment B91. The method of embodiment B90, wherein the first payload and the second payload are different.
- [0408] Embodiment B92. The method of any one of embodiments B86 to B91, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
- [0409] Embodiment B93. The method of embodiment B92, 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.
- [0410] Embodiment B94. The method of embodiment B92 or embodiment B93, 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.
- [0411] Embodiment B95. 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 B47 to B54, 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.
- [0412] Embodiment B96. The method of embodiment B95, wherein the nucleic acid is RNA or DNA.

- [0413] Embodiment B97. The method of embodiment B96, wherein the payload encodes a polypeptide.
- [0414] Embodiment B98. The method of any one of embodiments B95 to B97, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
- [0415] Embodiment B99. The method of any one of embodiments B95 to B98, 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.
- [0416] Embodiment B100. The method of embodiment B99, wherein the first payload and the second payload are different.
- [0417] Embodiment B101. The method of any one of embodiments B95 to B100, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
- [0418] Embodiment B102. The method of embodiment B101, 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.
- [0419] Embodiment B103. The method of embodiment B101 or embodiment B102, 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.
- [0420] Embodiment B104. The method of any one of embodiments B95 to B103, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.
- [0421] Embodiment B105. The method of embodiment B104, 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.

- [0422] Embodiment B106. The method of any one of embodiments B95 to B105, wherein the effective amount ranges from about 5 μ g to 1000 μ g.
- [0423] Embodiment B107. 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 B47 to B54, 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.
- [0424] Embodiment B108. The method of embodiment B107, wherein the nucleic acid is RNA or DNA.
- [0425] Embodiment B109. The method of embodiment B108, wherein the payload encodes a polypeptide.
- [0426] Embodiment B110. The method of any one of embodiments B107 to B109, 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.
- [0427] Embodiment B111. The method of embodiment B110, wherein the first payload and the second payload are different.
- [0428] Embodiment B112. The method of any one of embodiments B107 to B111, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
- [0429] Embodiment B113. The method of embodiment B112, 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.
- [0430] Embodiment B114. The method of embodiment B112 or embodiment B113, 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.

[0431] Embodiment B115. The method of any one of embodiments B107 to B114, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.

[0432] Embodiment B116. The method of embodiment B115, 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.

[0433] Embodiment B117. The method of any one of embodiments B107 to B116, wherein the effective amount ranges from about 5 μ g to 1000 μ g.

[0434] SEQUENCES

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Nucleic acid sequence of WT S (Wuhan strain) (from 5'-end to 3'-end)	cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaagggtgttagaagctccgtgctgcactct
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SEQ ID NO: 2 Protein sequence of WT S (Wuhan strain) (from N-terminus to C- terminus):	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVFCEQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRFTLLKYENNG TTDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNI TNLCPFGEVFNATRFASVYAWNKRISNCVADYSVLVNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNLDLSDKVGGNYNLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQGF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQTSNPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSPKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLCA QKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDK AHFPREGVFVSNGTHWFTVQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKE IDRLNEVAKNLNESLIDLQELGKYEYQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
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241
240

Nucleic acid sequence
of S-(deg-RBD) for
Wuhan strain (from 5'-
end to 3'-end)

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SEQ ID NO: 4 Protein sequence of S- (deg-RBD) for Wuhan strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVVYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCEFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRTFLLKYNNENG TITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPAEIVRFQ ITNLCPFGEVFOATRFASVYAWNKRKISNCVADYSVLNYSASFSTFK CYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEI YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELL HAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQ FGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLY QDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPGAGICASYQTQTSNPRRARSVASQSIIAYTMSLGAENSV YSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLQ YGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNF SQILPDPSPKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLIC AQKFNGLTVLPLLDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASAL GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQ IDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHG KAHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIV NNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNI KEIDRLNEVAKNLNESLIDLQELGKYEYIKWPWYIWLGFIAGLIIV MVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 5 Nucleic acid sequence of S-(deg-S2) for Wuhan strain (from 5'- end to 3'-end)	atgttctgttctctggctcctgctgctctgtgctcctcacagtgcgtcaatctgacaactcggactcagctgcc cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaaggctttagaagctccgtgctgactct acacaggatctgttctgccattcttagcaacgtgacctggtccacgccatccagctgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgctctttaaagatggcgtgtacttcgctctaccgagaagagcaac atcatcagaggctggatcttggcaccacactggactcgaagacacagctctgctgctgctgaacaatgcc ccaacgtgctcatcaagggtgctgaggttcagtttgaatgacccctcctggcgtgtactatcacaagaaca ataagagctggtgagtcgagtttagagtgtaftctagcgccaacaactgcacattgagtagctgagcca gccttctctgatggacctggaggcgaagcagggaattcaagaacctgagggagttcgtgttaagaatac gacggctactcaaaatctactctaagcacacecccatcaacctgggtgcgcgacctgcctcagggcttcagc gacctggagccctgggtgatctgctatcgcatcaacatcacccggttcagacactgctgacctgcac agaagctacctgacacccggcgaactccttagcggatggaccgcccgcgtgcgcctactatgtggcta cctcagcccccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggtgagccc tggacccctgagcgagacaaagtgtactgaagtccttaccgtggagaagggcatctatcagacatcca attcaggggtcagccaaccgagttctatcgtgcgcttcttaatacacaaacctgtgccatttggcaggtgt tcaacgcaaccgcttccagcggtgacgcctggaataggaagcgatcagcaactgcgtggcgcgactat agcgtgctgtacaactcgcctcttccagcactttaaagtgctatggcgtgtccccacaaagctgaatgacct gtgctttaccaacgtctacgcgattcttctgtatcaggggcgagaggtgcgacagatgcctccggcca gacaggcaagatcgagactacaattataagctgcagacgattcacccgctgctgctgctgacctggaacag caacaatctggtattccaaagtggcggaactacaattatctgtaccggctgtttagaagagcaatctgaag cccttcgagagggacatctctacagaaatctacaggccggcagcacccttgcattggcgtgagggctt

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SEQ ID NO: 6 Protein sequence of S- (deg-S2) for Wuhan strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCEFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRTFLLKYENNG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNI TNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNFGLTGTGVLTESNKKFLPFQGF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNS YECDIPIGAGICASYQTQTNPRRARSVASQSIIAYTMSLGAENSVAY SQNSIAIPTQFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQY

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for Wuhan strain (from 5'-end to 3'-end)	ataagagctggatggagtcgagtttagagtgattctagcgccaacaactgcacatttgagtagtgagcca gccttctctgatggacctggaggcgcaagcaggcgcaatttcaagaacctgaggagtgctgtttaagaatc gacggctacttcaaaatctacttaagcacaccccacacacctgggtgcgagacctgcctcagggcttcagc gocctggagccctggatctgctatcggcatcaacatcacccgggttcagacactgctggccctgcac agaagctactgcacccggcgactcctctagcggatggaccgcggtgcgctgcgctactatgtgggcta cctccagcccgaccttctctgaagtacaacgagaatggcaccatcacagacgcagtggttgctgcgc tgacccctgagcgagacaagtgtactgaagtcctttaccgtggagaaggcgatctatcagacatcca atttcagggtgcagccagcgagcgatcgtgcgcttctgaatcacaaacctgtgcccatttggcgagg tgttcgaggaacccgcttcgccagcgtgaacgctggaatagggaagcgatcagcaactgcgtggcgac tatagcgtgctgtacaactcgcctcttccagcaccttaagtctatggcggtgcgcacaaagctgaatgac ctgtgctttaccaacgtctacgcgattcttctgtatcagggcgacgaggtgcgcagatgcgcgcgcgc cagacaggcaagatcgcagactacaattataagctgcagacgatttcaccggctgcgtgatcgcctggac agcaacaatctggattcacaagtggcggaactacaattatctgtaccggctggttagaagagcaatctga agcccttcgagagggaatctctacagaaatctaccaggccggcgagcacccttgcgaatggcggtggaggc tttaactgttatttccactccagtcctacggcttcagcccacaacggcggtgggtatcagccttaccgctg gtggtgctgagctttagctgctgcagcccgcaacagtgctgcgcgcgaagagtcaccaatctggtg aagaacaagtgcgtgaattcaactcaacggcctgaccggcacagcggtgctgaccgagtcacaagaa gttctgctccatttcagcagttcggcgaggacatcgcagataccacagacgcggtgcgcgacccacagacc tgagatcctggacatcacacctgctcttctggcggtgagcgtgatcacacccggcaccataaagca accagggtggcggtgctgtatcaggacgtgaattgtaccgaggtgcccgtggctatccagccgatcagctga ccccacatggcggtgtacagcacggctcacaagcttccagacaagagccggatgctgatcggagc agagcacgtgaacaattcctatgagtgacatccaatcggcgccggcatctgtccttaccagacca gacaaactctccagaagagccggagcgtggcctccagctatcatcgcctataccatgctcctggcggc cgagaacagcgtggcctacttaacaatagcatcgccatcccaacaaactcacaatctctgtgaccacaga gatectgcccgtgtccatgaccaagacatctgtggactgcacaatgtatctgtggcgatttaccgagtgca gcaactgctgctccagtagcgagctttgtaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggaggtgttgcgcaggtgaagcaatctacaagacccccctatcaaggacttt ggcggttccaaatttccagatcctgctgatccatcaagccttcaagcggagctttatcaggacctgct gttcaacaaggtgacctggcgatgcggcttcacaaagcagtatggcgattgcctggcgacatcgagc cagggaactgatctgcgcgcagaagttaattggcctgaccgtgctgcacccctgctgacagatgagatgat cgcacagtacacaagcgccctgctggcggcaccatcacatccgatggaccttcggcgagagcgcc ctccagatcccccttgcctgagatggcctataggttcaacggcatcggtgaccagaatgtgctgtacg agaaccagaagctgatcgccaatcagtttaactcgcgcacggcaagatccaggacagcctgtcctctacag ccagcgccctgggcaagctcaggatgtgtgaatcagaacgcccaggccctgaataacctgtggaagca gctgagcagcaacttcggcgccatctctagcgtgctgaatgacatcctgagccggctggacaaggtggagg cagaggtgcagatcgaccgctgacaccggcggtcctcagagcctcagacctatgtgacacagcagct gatcaggcgcgcgagatcaggccagcgccaatctggcagcaaccaagatgtccgagtgctgctggg ccagtctaagagagtgactttgtggcaagggtatcaactgatgtccttcctcagctgccccacacggc gtggtgtttctgcacgtgacctacgtgcccgcggaggaagaacttaccacagcccctgccatctgccac gatggcaaggcccacttccaaaggaggcggtgttctgttccaacggcaccactgtttgtgacacagcg caattctacgagcccagatcacaccacagacaacacctctgtgagcggcaactgtgacgtggtcatcgg catcgtgaacaataccgtgtatgatcactccagcccgagctggacagctttaaggaggagctggataagtat ttcaagaatcacacctccctgacgtggtatctggcgacatcagcggcatcaatgcctcgtgtgtaacatcc agaaggagatcgaccgctgaacgaggtggctaagaatctgaacgagagcctgatcgacctccaggagct gggcaagtatgagcagtaacatgaagtgccctggatcatctggctgggttcacgcgcgctgatcgcct cgtgatgtgaccatcatgctgtctgtatgacatcctgctgttctgctgaagggtgctgtgagctgtggtc ctgctgtaagttgacgaggatgactctgaacctgtgctgaaggcggtgaagctgacttacacctaa
SEQ ID NO: 10	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDNPVLPFNDGVYFAS

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Protein sequence of S- (deg-RBD-801) for Wuhan strain (from N- terminus to C-terminus)	TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCEVFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRFTLLKYNNENG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPAEIVRFPO ITNLCPFGEVFAATRFASVYAWNKRISNCVADYSVLYNSASFSTFK CYGVSPTKLNLDLCTNVYADSFVIRGDEVRIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEI YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELL HAPATVCGPKKSTNLVKNKCVNFNENGLTGTGVLTESNKKFLPFQQ FGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLY QDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVA YSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQ YGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFQF SQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLIC AQKFNGLTVLPLLDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASAL GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQ IDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR VDFCGKGYHLSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDG KAHFPREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIV NNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQ KEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIV MVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 11 Nucleic acid sequence of S-(deg-RBD-1194) for Wuhan strain (from 5'-end to 3'-end)	atgttcgtcttcctggctctgctgctctgctgctcactgacagtcgctcaatctgacaactcggactcagctgcca cctgcttataactaatagcttcaccagaggcgtgtactatcctgacaagggttttagaagctccgctgctgactct acacaggatctgtttctgcatctttagcaacgtgacctggttccagccatecagctgagcggcaccatagg caaaaagggttcgacaatccctgctgctcttttaacgatggctgtacttgcctctaccgagaagagcaac atcatcagaggctgcatcttggcaccacactggactccaagacacagctctctgctgacgtgaacaatgcc ccaacgtggtcatcaagggtgctgaggttccagtttttaagtatccctcctggcgtgtactatcacaagaaca ataagagctggtgagtcgagtttagagtgattctagcgccaacaactgcacattgagtagctgagcca gccttctctgagtgacctggaggggcaggcgaattcaagaacctgagggaggtctgttttaagaatctc gacggctacttcaaaatctacttaagcacacccccatcaactggtgctgacgtgctcagggcttcagc gcccgtgagccctggtggtatctgctatcgccatcaacatcacccggttcagacactgctggtccctgac agaagctacctgacacccggcactccttagcggaatggaccgcccgtgctgcccactatgtgggcta cctccagcccccggacttctgctgaagtacaacgagaatggcaccatcacagacgcagtggtggtgccc tggaccccctgagcgagacaagtgtacactgaagtccttaccgtggagaaggcatctatcagacatecca atttcagggtgcagccagccgagcgtatcgtgcttctcgaatcacaacctgtgccatttggcgagg tggtcaggcaacccgcttcgacgctgtacgctggaataggaagcgatcagcaactgctggtgcccgcac tatagctgctgtacaactcgcctcttctcagcaacttaagtctatggcgtgtccccacaaagctgaatgac ctgtgctttacacagcttacgcgattcttctgctgacggggcgacgaggtgcccagatcggccccggc cagacaggcaagatcgagactacaattataagctgcagacgattcaccggctgctgctgacgctggaac agcaacaatctggattccaaagtggcgggcaactacaattatctgtaccgctgtttagaagaagcaatctga agcccttcgagaggacatctctacagaaatctaccaggcggcagcacccttgcaatggcgtggagggc tttaactgttattccactccagtcctacggcttcagcccacaaacggcgtgggtatcagccttaccgctg gtggtgctgagctttgagctgctgcagcccagcaacagtgctgcccgaagaagtcaccaatctggtg aagaacaagtgcgtgaactcaactcaacggcctgacggcacaggcgtgctgacaggagtcacaagaag gttctgctcatttcagcagttcggcaggacatcgagataccacagacgctgctgctgacccacagaccc

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SEQ ID NO: 12 Protein sequence of S- (deg-RBD-1194) for Wuhan strain (from N- terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIKVCDFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFK NLREFVFKNIDGYFKIYKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGDSSTSGWTAGAAAYVGYLQPRFTLLKYNENG TITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPAEIVRFPQ ITNLCPFGEVFPQATRFASVYAWNKRKISNCVADYSVLYNSASFSTFK CYGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEI YQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELL HAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQ FGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLY QDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQTSNPRRARSVASQSIIAYTMSLGAENSV YSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQ YGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFN SQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLIC AQKFNGLTVLPPLLTDEMIAQYTSALLAGTTTSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASAL

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	GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQ IDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDG KAHFPREGVVFVSNQTHWVFTQRNFYEPQIITDNTFVSGNCDVVIGIV NNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQ KEIDRLNEVAKNLQESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIV MVTIMLCCMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 13 Nucleic acid sequence of S-(deg-RBD-122- 165-234) for Wuhan strain (from 5'-end to 3'-end)	atgttcgtcttctcgttcctgctgctcctcgttctcctcacagtgcgtcaatcgcacaactcggactcagctgcca cctgcttataactagcttcaccagaggcgtgactatcctgacaagggttttagaagctccgtgctgcactct acacaggatctgtttctgccattctttagcaacgtgacctggtccacgccatccacgtgagcggcaccatgg cacaaggcgttcgacaatcccgctgctgcttttaacgatggcgtgtacttcgctctaccgagaaagcaac atcatcagaggctggatctttggcaccacactggactcaagacacagctctcgtcgtgacgtgaaccagcca ccaacgtggatcaagggtgtgcgaggttccagtttttaagatcccttctcgtggcgtgtactatcaangaaca ataagagctggatggagtcgagtttagagtgtattctagcgaaccagtgacattgagtacgtgagcca gccttctctgagtcgacctggaggcgaagcagggaattcaagaacctgaggaggtcgtgttaagaatac gacgctacttcaaatctactctaaacacaccccaacacgtggtgcgacacctgcctcagggttcagc gccctggagccctggtggtatcctatcggtacccagatcacccgggttcagacactgctggccctgcac agaagctacctgacacccggcgactcctctagcggatggacggccggcgtgcgcgctactatgtgggcta cctccagcccccgaaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggaftgcgcc tggacccctcgtgagcgagacaagtgctacactgaagtccttaccgtggagaaggcgtatctacagatcca attcaggggtgcagccaaccgagcgatcgtgcgttctcgaatcacaaacctgtgccatttggcgagg tgttcaggcaacccgcttcgccagcgtgtacgctggaaatagggaagcggatcagcaactgcgtggccgac tatagcgtgctgtacaactccgctctttagcacctttaagtctatggcgtgtcccccacaaagctgaatgac ctgtctttaccaacgtctacgccgattcttctgtatcagggcgacgaggtgcgcagatcgccccggc cagacaggcaagatcgcaactacaattataagctgcagacgattcacggctgctgtatcgccctggaac agcaacaatctggaatcgaagtgggcggaactacaattatctgtacggctgtttagaagagcaatctga agccctcagagaggacatctctacagaaatctaccaggcggcgacaccccttgaatggcgtggagggc tttaactgtatttccactcagctctacggcttcagcccacaaaggcgtgggctatcagccttaccgctg gtgggtgctgagctttgagctgtgcacgccccagcaacagtggtgcggccccagaagtccaccaatctgtg aagaacaagtgcgtgaacttcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaagaan gttctgccaattcagcagttcggcaggacatcgagataccacagacgcggtgcgcgacccaagaccc tggagatcctggacatcacacctgctcttctggcggtgagcgtgatcacacccggcaccatacaagca accaggtggccgtgctgtatcaggacgtgaattgtaccgaggtgccgtggtatccacgccgatcagctga ccccacatggcggtgtacagcacggctccaacgtcttcagacaagagccggatgcctgatcggagc agagcagtgacaattctatagtgacacatccaatcggcgccgcatctgtgctcttaccagacca gacaaactctccagaagagccggagcgtggcctccagctatcatcgctataacctgtccctgggcgc cgagaacagcgtggcctacttaacaatagcatcgccatcccaaccaactcacaatctctgtgaccacaga gatcctgcccgtgtccatgaccaagacatctgtgactgcacaatgtatatctgtggcgattctaccagtgca gcaacctgctgctccagtaaggcagctttgtaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggaggtgttgcgccaggtgaagcaatctacaagacccccctatcaaggacttt ggcggttcaattttccagatcctgctgatccatccaagccttcaagcggagctttatcaggacctgctg ttcaacaaggtagccctggcgatgcggcttcatcaagcagtatggcgattgctggcgacatcgagcc agggacctgatctgcgccagaagtttaattgacctgacctgtgcccacctgctgacagatgagatgac gcacagtacacaagcgccctgctggccggcaccatcacatccggatggaccttcggcgaggagccgccc tcagatccccttgcctgagatggcctatagggttaacggcagtcggcgtagccagaatgtgctgtacga gaaccagaagctgacgccaatcagtttaactcggcctatggcaagatccaggacagcctgctctacagc cagcgccctgggcaagctccaggatgtggtgaatcagaacgccaggccctgaataccctggtgaagcag ctgagcagcaacttggcgccatctctagcgtgctgaatgacatcctgagccggctggacaagggtggaggc agaggtgcagatcgaccgctgacacccggcctccagagcctccagacctatgtgacacagcagctg

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SEQ ID NO: 14 Protein sequence of S- (deg-RBD-122-165- 234) for Wuhan strain (from N-terminus to C- terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLD SKTQSLIVN[Q]ATNVVIK VCE FQFCNDPFLG VYYHKNNKSWMESEFRVYSSAN[Q]CTFEYVSQPF LMDLEGKQGNFK NLREFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGI[Q]IT RFQTLALHRSYLT PGDSSSGWTAGAAAYYVGYLQPRTFLLKY NEN GTITDAVDCALDPLSETKCT LKSFTVEKGIYQTSNFRVQP[A][E]IVRFP [Q]ITNLC PFGEVF[Q]ATRFASVYAWN RKISNCVADYSVL YNSASFSTF KCYGVSPTKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYK LPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTE IYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL SFELL HAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQ FGRDIADTTDAVRDPQTLEILDITPCSFGGVS VITPGTNTSNQVAVLY QDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDIPIGAGICASYQTQ TNSPRRARSVASQSIIAYTMSLGAEN SVA YSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTEC SNLLQ YGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNF SQILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLIC AQKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL SSTASAL GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAEVQ IDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKR VDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DG KAHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIV NNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASV VNIQ KEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIV MVTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPV LKGVKLHYT
SEQ ID NO: 15 Nucleic acid sequence of WT S (Delta strain) (from 5'-end to 3'-end)	atgttcgtcttctggtcctgctgctctggtctectcagtgcgctcaatctgcgaactcggactcagctcca cctgctatactaatagtctcaccagaggcgtgtactatcctgacaaggtgttagaagctcctgctgactct acacaggatctgtttctgccattcttagcaacgtgacctggttccagccateccagtgagcggcaccaatgg cacaagcgggtcgacaantccgctgctgctcttaacgatggcgtgtacttcgctctatcgagaagagcaac atcatcagaggctggatctttggcaccacactggactccaagacacagtctctgctgacgtgaacaatgcc ccaacgtggtcatcaaggtgtgcgagtccagtttgtaatgatccctcctggacgtgtactatcacaagaaca ataagagctggatggagtccggagtgtattctagcgcacaactgcacattgagtacgtgagccagcctt cctgatggacctggagggaagcagggaattcaagaacctgaggagggttcgtgttaagaatatcgacgg ctactcaaatctactctaagcacacccccatcaacctggtgcgcgacctgcctcagggtctcagcgccctg gagccctggtggatctgctatcggaatcaacatcacccgggttcagacactgtcggccctgcacagaagc

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SEQ ID NO: 16 Protein sequence of WT S (Delta strain) (from N-terminus to C- terminus)	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLDSTQSLIVNNAITNVVIKVCEFQFCNDPFLDV YYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFNKLR EFVFKNIDGYFKIYSKHTPINLVRDLPGQFSALEPLVDLPIGINITRFQT LLALHRSYLPDSSSGWTAGAAAYYVGYLQPRFTLLKYNNENGTTT DAVDCALDPLSEKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNL

252
251

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SEQ ID NO: 17 Nucleic acid sequence of WT S (Wuhan strain S-2P strain) (from 5'- end to 3'-end)	atgttcgtcttctggtcctgctgctctggtctctctcacagtgcgtcaatctgacaactcggactcagctcca cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaagggtgttagaagctccgtgctgcaact acacaggatctgttttgcattcttagcaactggaactggttccacgccatccacgtgagcggcaccatgg cacaagcgggtcgacaatcccgctgctctttaaactgagcgtgtacttcgectctaccgagaagagcaac atcatcagaggctggtatcttggcaccacactggactccaagacacagtctctgctgacgtgaacaatgcc ccaactggtgcatcaagggtgtgaggttcagtttgaatgacccctctggcgtgtactatcacaagaaca ataagagctggatggagtcgagtttagagtgattctagcgcacaactgcacattgagtagctgagcca gcttctctgagtgacctggagggaagcagggaattcaagaacctgagggaggtcgtgttaagaatac gacggctacttcaaaatctactctaacgacaccccacatcaacctggtgctgacgtgctcagggttcagc gcccgtggagccctggtggtatctgctatcggcatcaacatcccggttcagacactgctggccctgcac agaagctacctgacacccggcgactctctagcggatggaccgcggcgctgcccactactatgtggccta ctccagccccggacctctctgaagtacaacgagaatggcaccatcacagacgcagtggttgctgccc tggacccctgagcgagacaaagtgtactgaagtccttaacctggtgagaagggcatctatcagacatcca attcagggtgcagccaaccgagctatctgtgcttctcaatatacaaacctgtgcccatttggcgaggtgt tcaacgcaaccgcttcgacagcgtgtacgcttgaatagggaagcggatcagcaactgcgtgcccactat agcgtgctgtacaactccgctcttctcagcaccttaagtgtatggcgtgtccccacaaagctgaatgacct gtgctttaccaacgtctacgcgattcttctgtagcaggggcgacgaggtgcgccagatcgccccggcca gacaggcaagatcgagactacaattataagctgcagacgattcaccggctgctgtagcctggaacag caacaatctggattcaaaagtggcggaactacaattatctgacggctgtttagaagagcaatctgaag cccttcgagagggaatctctacagaaatctaccaggccggcagaccccttgaatggcgtggagggttt aactgttatttccactccagtcctacggcttcagcccacaaacggcgtggctatcagccttaccgctggt ggtgctgagctttgagctgtgcagccccagcaacagtggtgcggccccagaagtcaccaatctggtga agaacaagtgcgtgaactcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaagaagaa gttctgccaattcagcagttcggcagggaatcgacagataccacagacgcggtgcgacccacagaccc tggagatcctggacatcacacctgtctcttctggcggtgagcgtgacacacccggcaccatataagca accagggtggccgtgctgtatcaggacgtgaattgtacgaggtgcccgtggtatccacgcccagatcagctga ccccaatggcgggtgtacagacccgctccaacgtcttcagacaagagccggatgctgtagcggagc agagcacgtgaacaattctatgagtgacatcccaatcggcggcgcatctgtgctcttaccagacca gacaaactctccagaagagccggagcgtggcctccagctatcatcgctataccatgtccctgggcgc cgagaacagcgtggcctactctaaacatagcatcgccatcccaaccaattcacaatctctgtgaccacaga

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SEQ ID NO: 18 Protein sequence of WT S (Wuhan strain S-2P strain) (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVFCEQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRTFLLKYENENG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNI TNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLYNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQGF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPLLTDemiaQYTSALLAGTTTSGWTFGAGAAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAIVSLNDILSRLDPPAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHGDK AHFPREGVFVSNGTHWFTVQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKE

	IDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 19 Nucleic acid sequence of WT S (Delta S-2P strain) (from 5'-end to 3'-end)	atgttcgtcttcctggctcgtcctctggctcctcacagtcgtcaatctgcgaactcggactcagctgcca cctgcttataactaagcttcaccagaggcgtgtactatcctgacaagggttttagaagctccgtgctgcaet acacaggatctgtttctgccattcttagcaacgtgacctgggtccacgccatccacgtgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgcttttaacgatggcgtgtacttcgctctatcgagaagagcaac atcatcagaggctggatctttggcaccacactggactccaagacacagtcctctgctgacgtgaacaatgcc ccaacgtggctcatcaagggtgtgcgagttcagttttgtaatgacccctcctggacgtgtactatcacaagaaca ataagagctggatggagtcggagtgattctagcgcacaactgcacatttgagtagctgagccagccttt cctgatggacctggaggcgaagcaggcgaattcaagaacctgagggtgctgttttaagaatafcgacgg ctacttcaaatctacttaagcacacccccatcaacctgggtgcgcgacctgcctcagggttcagcgccctg gagccctgggtggtctgctatcggcatcaacatcacccgggttcagacactgctggccctgcacagaagc tacctgacacccggcactcctctagcggatggaccgcccggcgtgcgcctactatgtgggtacttcca gccccggaccttcctgctgaagtacaacgagaatggcaccatcacagacgcagtggttgccctggacc ccctgagcgcagacaagtgtagactgaagtcccttaccgtggagaaggcgcctatcagacatccatttcag gggtgcagccaaccgagctatcgtgcgtttccataatcacaaacctgtgccatttggcgagggttcaacg caaccgcttcgcagcgtgtacgcctggaataggagcggatcagcaactgcgtggccgactatagcgtg ctgtacaactccgctctttcagcacctttaagtctatggcgtgtccccacaaagctgaatgacctgtcttta ccaacgtctacgcgattcttctgtatcaggggcgacgagggtgcgcagatgcgccccggccagacagg caagatcgagactacaattataagctgccagacgatttcaccggctgcgtgacgcctggaacgacaaca tctggattccaaagtggcggaactacaattatcgggtaccggctgttttagaaagagcaatctgaagcccttc gagagggacatctacaganaatctaccaggccggcagcaagccttgcaatggcgtggagggtcttaactgt tatttccactccagtcctacgggttcagcccaaacggcgtgggtctatcagccttaccggtgggtgct gagcttgagctgctgcacgccccagcaacagtggtgcggccccagaagtccaccaatctgtgaagaaca agtgcgtgaactcaactcaacggcctgacggcacaggcgtgtgacccgagtcacaagaagtctgtc catttcagcagttcggcaggacatcgagataccacagacgcgtgcgcgacccacagaccctggagat cctggatcacacccctgctcttccggcggtgagcgtgatcacaccggcaccaatacaagcaaccagg tggccgtgctgtatcaggcgtgaattgtacagggtgccgtgtgtatccacgcgacagctgaccccaa catggcgggtgtacagacccggctcaacgtcttcagacaagagccggatgctgtatcggagcagagca cgtgaacaattcctatgagtgcacatcccaatcggcgccggcactgtgctcttaccagaccagacaaa ctctgcagaagagcccgagcgtggcctccagctatcatcgctataccatgtccctggcgccgagaa cagcgtggcctacttaacaatagcatgcacccaaccaactcaaatctctgtgaccacagagatcctg ccggtgtccatgaccaagacatctgtgactgcacaatgtatatctgtggcgattctaccgagtgacgaacct gtgtctccagtcaggcagctttttgaccagctgaatagagccctgacaggcatcgccgtggagcaggataa gaacacacaggaggtgttcgccagggtgaagcaaatctacaagacccccctatcaaggactttggcggtt caatttttccagatcctgctgacatccaagccttcaagcggagctttatcaggagctgctgttcaaca gggtgacctggcggatgcgggttcacaaagcagatggcgattgcctggcgacatcgagccagggacc tgatctgcgcccagaagttaattggcctgaccgtgtgccaacctgctgacagatgagatgacgcacagta cacaagcgcctgtcggccggcaccatcacatccggtgacctcggcgaggagcgcgcctccagatc cccttgcctgagatggcctataggttcaacggcatcggcgtgaccagaatgtgctgtacgagaaccag aagctgacgccaatcagtttaactcggccatcggcaagatccaggacagcctgtcctctacagccagcgc ctgggcaagctccagaatgtgtgaatcagaacgccaggccctgaataacctggtgaagcagctgagca gcaacttcggcgccatctctagcgtgctgaatgacatcctgagccggctggacccggaggcagaggt gcagatcagccggtgatcaccggccggctccagagcctccagacctatgtacacagcagctgatcagg gcccggagatcaggccagcgcgaatctggcagcaaccaagatgtccgagtgctgctggccagtcta agagagtggaacttttggcaagggtatcaccgtatgtcttccctcagtcgtccccacaggcgtgtgttt ctgcagtgacctacgtgccgcccaggagaagaacttcaccacagccctgcctctgccaagatggcaa ggcccactttcaaggagggtgtgtgttccaacggcaccactggtttgtacacagcgaatttctac gagcccagatcacacagacaacacctctgtgagcggcaactgtgacgtgtgtatcggcatcgtgaa caataccgtgtatgatccactccagcccgagctggacagctttaaggaggagctggataagtattcaagaat

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SEQ ID NO: 20 Protein sequence of WT S (Delta strain S- 2P strain) (from N- terminus to C-terminus)	MFVFLVLLPLVSSQCVNLRTRTLPPAYTNSFTRGVVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLD SKTQSL L VNNATNVVIKVCEFQFCNDPFLDV YYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFNKLR EFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT LLALHRSYLT PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTTT DAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNL CPFGEVFNATRFASVYAWNKRKRISNCVADYSVLVNSASFSTFKCYG VSPTKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKL PDD FTGCVIAWNSNNLDSKVGGNYNRYRLFRKSNLKPFERDISTEIYQA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSELLHAP ATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGR DIADTTDAVRDPQTL EILDITPCSFGGVSVITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSE CDIPIGAGICASYQTQTSRRRARSVASQSIIAYTMSLGAENSVAYS NSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTEC SNLLQYGS FCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQIL PDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLICAQK FNGLTVLPPLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKL QNVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPAEVQIDR LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF CGKGYHLMSPQSA PHGVVFLHVTYVPAQEKNFTTAPAICHGDKAH FPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNT VYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASVVNIQKEID RLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTI MLCCMTSCCCLKGCCSCGSCCKFDEDDSEPV LKGVKLHYT
SEQ ID NO: 21 Nucleic acid sequence of S-(deg-RBD) for Delta strain (from 5'- end to 3'-end)	atgttcgtcttctggtctctgctgctctggtctctcacagtgcgtcaatctcgaaactcggactcagctcca cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaagggtgttagaagctccgtgctgactct acacaggatctgtttctgccattcttagcaacgtgacctggttcacgccatccacgtgagcggcaccaatgg cacaagcgggttcgacaatcccgctgctgcttttaacgatggcgtgtacttcgctctatcgagaagagcaac atcatcagaggctggtatctttggcaccacactggactccaagacacagctctgctgacgtgaacaatgcc ccaacgtggtcatcaagggtgtgaggttcagttttgtaatgacccctcctggacgtgtactatcacaagaaca ataagagctggatggagtcggagtgattctagcgccaacaactgcacattgagtagctgagccagccttt cctgatggacctggagggaagcagggaattcaagaacctgaggaggttcgttttaagaatatcgacgg ctactcaaaatctactetaagcacacccccatcaacctggtgcgcgacctgcctcagggttcagcgcctg gagccctggttgatctgctatcggeataacatcaeccggttcagacactgctggccctgcacagaagc tacctgacacccggcgactcctctagcggatggaccgcccgcgtgcgcgctactatgtgggtacctcca gccccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggattgcgccttgacc ccctgagcgagacaaagtgtacactgaagtcctttaccgtggagaagggaatctatcagacatccaatttcag ggtgcagccagccgagggatcggtgcgtttcctgaaatcacaaacctgtgccatttggcgagggttcga ggcaacccgcttcgcagcgtgtacgcctggaataggaagcggatcagcaactcgtggccgactatagc gtgctgtacaactcgcctcttcagcaccttaagtgtatggcgtgtccccacaaagctgaatgacctgtgc tttaccacgtctacgcgattcttctgtatcagggggcgacgaggtgcgcagatgcgcccgccagaca

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SEQ ID NO: 22 Protein sequence of S- (deg-RBD) for Delta strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVS GTNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVCEFQFCNDPFLDV YYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFNKLR EFVFKNIDGYFKIYSKHTPINLVRDL PQGFSALEPLVDLPIGINITRFQT LLALHRSYLT PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNNENGTT DAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPAEIVRFPQITN LCPFGEVVFQATRFASVYAWNKRISNCVADYSVLVNSASFSTFKCYG VSPTKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDD FTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKPFERDISTEYQA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSELLHAP ATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQFGR DIADTTDAVRDPQTLEILDITPCSFGGVS VITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTG SNVFQTRAGCLIGA EHVNNSE

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256

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SEQ ID NO: 23 Nucleic acid sequence of S-(deg-RBD) for Wuhan S-2P strain (from 5'-end to 3'-end)	atgttcgtcttctggtctgctgctctggtctctcacagtgcgtcaatctgacaactcggactcagctgcca cctgcttatactaatagcttcaccagaggcgtgtaactatcctgacaagggttttagaagctccgtgctgactct acacaggatctgtttctgccattcttttagcaacgtgacctggttcacgccatccacgtgagcggcaccatgg cacaagcgggttcgacaatcccggtgctgcttttaacgatggcgtgtaactcgcctctaccgagaagagcaac atcatcagaggctggatctttggcaccacactggactcgaagacacagctctctgctgacgtgaacaatgcc ccaacgtggtcatcaagggtgctgaggttccagttttgtaatgatcccttctggcgtgtaactacacaagaaca ataagagctggatggagtcgaggttagagtgattctagcgcacaacactgcacatttagtagctgagcca gccttctctgatggacctggaggcgaagcaggcgaattcaagaacctgaggaggttcgtgttaagaatac gacggctacttcaaatctactctaaagcacacccccatcaacctgggtgcgcacctgcctcagggcttcagc gacctggagccctggtggtctgctatcggcatcaacatcacccggttcagacactgctggccctgcac agaagctacctgacacccggcgactcctctagcggatggaccgcccgcgtgcgcctactatgtgggcta cctcagccccggaccttctctgtaagtacaacgagaatggcaccatcacagacgcagtggttgctgccc tggacccctgagcgagacaaggtgtactgaagtcctttaccgtggagaaggcgatctatcagacatcca atttcagggtgcagccagcgcaggcgatgctgctgcttctgaaatcacaacctgtgccatttggcgaggt gttcgaggcaacccgcttcgacagcgtgtacgcctggaatagggaaggatcagcaactgcgtggccgact atagcgtgctgtacaactcgcctcttctcagcaccttaagtgtatggcgtgtccccacaaggctgaatgac ctgtgctttaccaacgtctacgccgattcttctgtatcagggcgacgaggtgcgcagatcgccccggc cagacaggcaagatcgagactacaattataagctgcagacgatttcaccggtgcgtgtatcgcttgaaac agcaacaatctggattccaaagtggcgccgaactacaattatctgtaccggctgtttagaagaagcaatctga agcccttcgagagggaatctctacagaaatctaccaggccggcagcacccttgcaatggcgtggaggggc tttaactgtatttccactcagctctacggcttcagcccacaacggcggtgggtatcagccitaccggtg gtggtgctgagctttgagctgctgacgccccagcaacagtgtgcggccccagaagtcaccaatctggtg aagaacaagtgcgtgaactcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaagaaga gttctgctcatttcagcagttcggcaggacatcgagataccacagacgcgtgcgcgaccacagacc tggagatcctggacatcacacctgctcttctggcggtgagcgtgatcacaccggcaccatacaagca accagggtggcgtgctgtatcaggacgtgaattgtaccgaggtgcccgtggtatccacgcatcagctga ccccaatggcggtgtacagaccggctccaacgtcttcagacaagagccggatgctgatcggagc agagcagctgaacaattctatgagtgagatccaatcggcgcggcatctgtgctcttaccagacc gacaaactctccagaagagccggagcgtggcctccagctatcatcgctataaccatgctcctggcg cgagaacagcgtggcctactcaacaatagcatcgccatcccaaccaactcacaatctctgtgaccacaga gatcctgcccgtgtccatgaccaagacatctgtgactgcacaatgtatactgtggcgattctaccgagtgca gcaacctgctgctccagtagggcagctttgtaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggaggtgttgcggcagggtgaagcaaatctacaagacccccctatcaaggacttt ggcggcttcaattttccagatcctgctgacatccaagccttcaagcggagctttatcaggagcctgctg ttcaacaaggtagacctggccgatgcggcttcacaaacagctatggcgattgcctggcgacatcgagcc agggacctgatctgcgcccagaagttaattggcctgaccgtgctgacacctgctgacagatgagatgac gcacagtacacaagcgccctgctggcgccaccatcacatccgagtgacattcggcgaggagccgccc

SEQ ID NO: 24 Protein sequence of S-(deg-RBD) for Wuhan S-2P strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVVYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIKVCEFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRLDPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRFTLLKYNENG TTTDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPAEIVRFPQI TNLCPFGEVFQATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSPKSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPLLTDEMIAQYTSALLAGTTTSGWTFGAGAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDK AHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVUNIQKE IDRLNEVAKNLNESLIDLQELGKYEYQIKWPWYIWLGFIAGLIAVM VTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 25 Nucleic acid sequence of S-(deg-RBD) for	atgttcgtcttcctggtcctgctgctcctggtctctcctcagtgctgaatctgcgaactcggactcagctgcca cctgctctatactaatagcttcaccagaggcgtgtaactactcagacaagggtttgagaagctccgtgctgactct acacaggatctgtttctgccattctttagcaacgtgacctggttcacgccaacacgtgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgctcttttaacgatggcgtgtacttcgctctatcgagaagagcaac atactcagaggctggatctttggcaccacaactggactcaagacacagctctctgctgactgtgaacaatgcca ccaacgtgtgctcaagaaggtctcgaatttcagtttttaagatcccttctctgacctctactatcacagaaca

Delta S-2P strain (from 5'-end to 3'-end)	ataagagctggatggagtcggagtgattctagcgccaacaactgcacafftgagtacgtgagccagcctt cctgatggacctggagggaagcagggaattcaagaacctgaggagtgctgtttaagaatatcgacgg ctactcaaatctacttaagcacacccccatcaacctggcgcgacctgctcagggcttcagegacctg gagccctgggtgafctgctatcggaatcaatcacccgggttcagacactgctgcccctgcacagaagc tacctgacacccggcgactcctctagcggatggacccggcgctgcccctactatgtgggtacctcca gccccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggaattgcgcccctggacc cctgagcgagacaaagtgtactgaagtcctttacctggagaagggcatctatcagacatccaatttcag ggcgagccagccgagggcgatcgctgctttcctgaaatcacaaacctgtgccafftggcgagggttcga ggcaaccgcttcgcccagcgtgtacgctggaatagggaagcggaatcagcaactgctggccgactatagc gtgctgtacaactccgctcttcagcaccttaagtgtatggctgttccccacaaagctgaatgacctgtgc tttaccacgtctacgcccgaftcttctgtatcaggggcgaggggtgcgacagatgcgcccggccagaca ggcaagatcgagactacaattataagctgacagacgatttcacggctgctgtatgcgctggaacgacaac aatctggattccaagtggcggaactacaattatcggtaccggctgtttagaagagcaatctgaagccctt cgagaggacatctctacagaatctaccaggccggcagcaagccttgcaatggcggtggagggtttaact gttattccacaccagtcctacggcttcagcccacaaaggcggtgggtatcagccttaccgctggtgtgt gctgagctttgagctgtgcacgcccagcaacagtgctggcccagaagtcaccaatctggtgaaga acaagtgcgtgaactcaactcaacggcctgaccggcacaggcggtgctgacggagtcacaagaagttc ctgcccattcagcagttcggcaggacatcgagataccacagacggcgtgcgacccacagacctgg agatcctggacatcacacctgctcttctggcggtgagcggtgatcacaccggcaccatacaagcaacc agggtggcggtgctgtatcaggcggtgaattgtaccgaggtgcccgtggctatccagccgacagctgaccc caacatggcggtgtacagcaccggctcacaactcttcagacaagagccggtgctgacggagcaga gcacgtgaacaattcctatgagtgacatcccaatcgccggcgatctgtgctcttaccagaccagac aaactctgcagaagagcccgagcggtggcctccagctatcatcgctataccatgtccctggcgccga gaacagcggtgctactcaacaatagcatcgccatcccaaccaacttcacaatctctgtacacagagatc ctgcccgtgctcatgaccaagacatctgtgactgcacaatgtatatctgtggcattctaccagtgacaa cctgtgctcagtaaggcagctttgtaccagctgaatagagccctgacaggcatcgccgtggagcagga taagaacacacaggaggtgttcgcccaggtgaagcaaatctacaagacccccctatcaaggactttggcg gttcaattttccagatcctgctgacatcccaagccttcaagcggagctttatcgaggacctgctgttcaa caaggtagccctggcgatcgccgcttcacaaagcagtatggcgattgctggcgacatcgagccaggg acctgatctgcgcccagaagttaattggcctgaccgtgctgcccacctgctgacagatgagatgacgac agtacacaagcgccctgctggcggcaccatcacatccggatggacctcggcgagggagccgcccica gatcccccttgcctatgagatggcctatagggtcaacggcatcgcgctgaccagaatgtgtgtacgagaac cagaagctgatcgccaatcagtttaactccgcatcggaagatccaggacagcctgctctacagccagc gcccctgggcaagctcagaatgtggtgaatcagaacgcccaggccctgaataccctggtgaagcagctga gcagcaatttcggcgccatctctagcgtgctgaatgacatcctgagccgctggacctggcgaggcaga gggtcagatcgaccgctgacccggcggctccagagcctccagacctatgtacacagcagctgatca gggcccggagatcaggggcagcgccaatctggcagcaaccaagatgtccgagtgctgtgtggccagt ctaagagagtggaactttgtggcaagggtatcactgatgtcttccctcagctgcccacacggcgtgtgt ttctgcagtgactacgtgcccggcaggagaagaacttcaccacagccccctgcatctgcccagatggc aaggcccactttcaaggaggggcggtgttcgtgtccaacggcaccactggttgtgacacagcgcaattct acgagccccagatcatcacacagacaacaccttcgtgagcggaactgtgacgtgtgcatcgccatctgt aacaataccgtgtatgatccactccagcccgagctggacagctttaaggaggagctggataagtattcaaga atcacacctccccgtgacgtggtatggcgacatcagcgcatcaatgcctccgtggtgaacatccagaagg agatcgaccgctgaacgaggtggctaagaatctgaacgagagcctgatcgacctccaggagctgggcaa gtatgagcagtaacatcaagtggcctgtgacatctggctgggtctcatcgccggcctgatcgccatctgtatg gtgacctatctgtgtgtgtatgacatcctgtgttctgctgaagggtgtgtgtatgtgtgtgtgtgtgt agtttgacgaggtgactctgaacctgtgtgaaggcggtgaagctgcaftacaccta
SEQ ID NO: 26	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLDSKTQSLIIVNNATNVVIK VCEFQFCNDPFLDV

Protein sequence of S-(deg-RBD) for Delta S-2P strain (from N-terminus to C-terminus)	YYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLR EFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT LLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTTT DAVDCALDPLSEKCTLSFTVEKGIYQTSNFRVQPAEAIVRFPQITN LCPFGEVFQATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYG VSPTKLNLDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDD FTGCVIAWNSNNLDSKVGNNYNYRYRLFRKSNLKPFERDISTEIIYA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAP ATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGR DIADTTDAVRDPQTLEILDITPCSFSGGVSVITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSE CDIPIGAGICASYQTQTSRRRARSVASQSIIAYTMSLGAENSVAYS NSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGS FCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQIL PDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICAQK FNGLTVLPPLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKL QNVVNQNAQALNTLVKQLSSNFGAIISSVLNDILSRDPPAEVQIDR LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF CGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHGDKAH FPREGVFSNGTHWVFTQRNFYEPQIITDNTFVSGNCDVVIGIVNNT VYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVUNIQKEID RLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTI MLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 27 Nucleic acid sequence of S-(deg-S2) for Delta strain (from 5'-end to 3'-end)	atgttcgtcttcctggtcctgctgctcctggtctcctcacagtcgctcaatctcggaactcggactcagctgcc cctgcttataactaagcttcaccagaggcgtgtactatcctgacaagggttttagaagctccgtgctgactct acacaggatctgtttctgcatctcttagcaacgtgacctggttccacgcatccacgtgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgctcttaacgatggcgtgtacttcgctctatcgagaagagcaac atcatcagaggctggatctttggcaccacactggactccaagacacagctctctgctgacgtgaacaatgcca ccaacgtggtcatcaagggtgctgaggttcagttttgtaatgacccctctgagcgtgtactatcacaagaaca ataagagctggatggagtgctggaggttattctagcggcaacaactgcacattgagtagcgtgagccagcctt cctgatggacctggagggaagcagggaatttcagaacctgaggagggttcgtgttaagaatatcgacgg ctacttcaaatctactetaagcacaccccatcaacctgggtgctgacgtgctcagggcttcagcgccctg gagccctgggtggtctgctatcggtcatcaacatcaaccgggttcagacactgctggccctgcacagaagc tacctgacacccggcactcctctagcggatggacccggcgtgcccctactatgtggctacctcca gccccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggttcgcccctggacc ccctgagcgagacaaagtgtactgaagtccttaccgtggagaaggcatctatcagacatcaatttcag ggtgcagccaaccgagctatcgtgcttcttaatatcacaacctgtgccatttggcgaggtgttcaacg caaccgcttcgacagcgtgtacgctgggaatagggaagcggatcagcaactgcgtggccgactatagcgtg ctgtacaactccgctcttccagcacctttaaagtgtatggcgtgtccccacaaagctgaatgacctgtgttta ccaacgtctacgctgattcttctgctacagggcgacgaggtgcgcagatcgcgcccgccagacagg caagatcgagactacaattataagctgacagacgatttcacggctgctgctgacgctggaacagcaaca tctggattccaaagtggcggaactacaattatcggtacccgctgtttagaagagcaatctgaagcccttc gagagggaatctctacagaaatctaccaggccggcagcaagccttgcaatggcgtggagggtttaaactgt tatttccactccagtcctacggcttcagcccacaacggcgtgggtatcagccttaccgctggtggtgct gagctttgagctgctgcagccccagcaacagtgctggccccagaagtcaccaatctggtgaagaaca agtgcgtgaactcaactcaacggcctgacccgacagcgctgctgacccagtcacaagaagtctctgc catttcagcagttcggcaggacatcgagataccacagacccgtgctgcgacccacagaccctggagat cctggacatcacacccgtctcttctggcggcgtgagcgtgatcacacccggcaccatacaagcaaccagg tgccgtgctgtatcaggcgtgaattgtaccgaggtgcccgtgctatccacgccgatcagctgaccccaa

261
260

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SEQ ID NO: 28 Protein sequence of S- (deg-S2) for Delta strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVVYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSFNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIVCEVFQFNDPFLDV YYHKNNKSWMESGVYSSANNCTFEYVSQPFMLDLEGKQGNFKNLR EFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT LLALHRSYLTGPDSSSGWTAGAAAYYVGYLQPRFTLLKYNNENGTTT DAVDCALDPLSEKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNL CPFGEVFNATRFASVYAWNKRKISNCVADYSVLYNSASFSTFKCYG VSPTKLNLDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLDD FTGCVIAWNSNNLDSKVGNYNYRYRLFRKSNLKPFERDISTEYQA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAP ATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQFGR DIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSE CDIPIGAGICASYQTQTSRRRARSVASQSIIAYTMSLGAENSVAYSQ NSIAIPTQFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGS FCTQLNRALTGIAVEQDKNTQEVFAQVKQYKTPPIKDFGGFQFSQIL PDPSKPSKRSFIEDLLFNKVTLDAGFIKQYGDCLGDIAARDLCAQK FNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKL QNVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDR LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF CGKGYHLMSPQSAHPGVVFLHVTYVPAQEKQFTTAPAICHGDKAH

	FPREGVVFVSQGTHWVFTQRNFYEPQIITDNTFVSGNCDVVIGIVQNT VYDPLQPELDSFKEELDKYFKQHTSPDVDLGDISGIQASVUNIQKEID RLNEVAKNLQESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTI MLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 29 Nucleic acid sequence of S-(deg-S2) for Wuhan S-2P (from 5'- end to 3'-end)	atgttcgtcttcctggctcgtcctctggtctctcacagtgcgtcaatctgacaactcggactcagctgcca cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaagggttttagaagctccgtgctgcaact acacaggatctgtttctgccattcttagcaacgtgacctggtccacgccatccacgtgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgcttttaacgatggcgtgtactctgacctaccgagaagagcaac atcatcagaggctggtatctttggcaccacactggaactcaagacacagctctgctgacgtgaacaatgcc ccaacgtggtcatcaagggtgctgaggtccagttttgtaatgatcccttctggcggtgtactatcacaagaaca ataagagctggatggagtcgagtttagagtgattctagcgccaacaactgcacattgagtagctgagcca gctttctgctgacgtggaggggcagcagggcaattcaagaacctgaggaggtctgttgaagaatac gacggctacttcaaaatctactaagcacacccccatcaacctggtgcgcgacctgctcagggcttcagc gcccgtggagccccgtggatctgctatcggcatcaacatcacccggtttcagacactgctggccctgcac agaagctacctgacacccggcgactccttagcggatggaccgcccgcgtgcgcgctactatgtgggcta cctccagccccggacctctgctgaagtacaacgagaatggcaccatcacagacgagtgattgcgccc tgaccacctgagcgagacaaggtgtactgaagtcctttaccgtggagaaggcgatctatcagacatcca atttcagggtgcagccaaccgagctctatcgtgcgctttcctaatacacaaacctgtgccatttggcgagggt tcaacgcaaccgcttcgcagcgtgtacgctggaatagggaagcggatcagcaactgcgtggcgcgactat agcgtgctgtacaactcgcctctttcagcaccttaagtctatggcgtgtcccacaaagctgaatgacct gtgctttacacagctctacgccattcttctgtatcagggcgacgagggtgcgcgagatgcgcccgcca gacaggcaagatcgcagactacaattataagctgcagacgatttcacggcgtgcgtgacgcttggaaacag caacaatctggattcacaagtgggcgcaactacaattatctgtaccgctgttttagaaagagcaatctgaag ccctcgagaggacatctctacagaaatctaccaggccggcagcacccttgcattggcgtggagggttt aactgttatttccactcagctctacggcttcagcccacaaacggcgtgggctatcagccttaccgctggt gggtgctgagctttgagctgctgcacgcccagcaacagtgtgcggcccaagaagtcaccaatctgtga agaacaagtgcgtgaactcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaacaagaa gttctgaccttcagcagttcggcaggacatcgagataccacagacgcgtgcgcgacccacagaccc tgagatcctggacatcacacctgctctttcggcgggcgtgagcgtgacacccggcaccaatacaagca accagggtggcgtgctgtatcaggacgtgaattgtacgaggtgccgtggtatccacgcccgatcagctga ccccaatggcggtgtacagaccggctccaaactcttcagacaagagccggatgcctgacggagc agagcacgtgaacaattctatgagtgacatcccaatcggcgccgcatctgtccttaccagacca gacaaactctccagaagagcccgagcgtggcctccagctatcatcgcctataccatgtccctggcgcc cgagaacagcgtggcctactctcagaatagcatcgcctcccaaccagttcacaatctctgtgaccacaga gatctgcccgtgctcatgaccaagacatctgtggactgcacaatgtatatctgtggcattctaccagtgca gcaacctgctgctcagtaggcagctttttaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggaggtgttcgcccaggtgaagcaaatctacaagacccccctatcaaggacttt ggcggttccaatttccagatcctgctgacccatcaagccttcaagcggagctttatcaggacctgct gttcaacaaggtgacctggccgatgccggttcatcaagcagtatggcgattgcctggcgacatcgagc cagggacctgactgcgcccagaagttaattggcctgaccgtgctgccacctgctgacagatgagatgat cgcacagtacacaagcgccctgctggcggcaccatcacatccggtgaccttcggcgaggagcggcc ctccagatccccctttccatgcagatggcctatagggtcaacggcatcgccgtgaccagaatgtgctgtacg agaaccagaagctgatgcgaatcagtttaactcgcctacggcaagatccaggacagcctgtcctctacag ccagcgccctgggcaagctccaggatgtggtgaatcagaacgcccaggccctgaataacctgtggaagca gctgagcagcaactcggcgccatctctagcgtgctgaatgacatcctgagccgctggacctggaggg cagagggtcagatcgaccggctgacaccggccggctccagagcctccagacctatgtacacagcagct gatcaggcgcccgagatcaggggcagcgcaatctggcagcaaccaagatgctcgagtgctgctggg ccagtctaagagagtgactttgtggcaagggtatcacctgatgtcctccctcagctgctccacacggc gtgggtttctgcagtgacctacgtgcccggcaggagaagcagttcacacagccccctgacctgctccac gatggcaaggcccacttccaaggaggggcgtgttcgtgtccagggaacccactggtttgtacacagcg

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SEQ ID NO: 30 Protein sequence of S- (deg-S2) for Wuhan S- 2P (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITR FQTLALHRSYLTGPDSSSGWTAGAAAAYVGYLQPRTFLLKYNENG TTDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNI TNLCPFGEVFNATRFASVYAWNKRKISNCVADYSVLVNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNVNYLYRFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQQF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAY SQNSIAIPTQFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFQFS QILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPLLTDemiaQYTSALLAGTITSGWTFGAGAAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPPEAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLSFPQSAPHGVVFLHVTYVPAQEKQFTTAPAICHDKG AHFPREGVFVSQGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVQ NTVYDPLQPELDSFKEELDKYFKQHTSPDVLGDISGIQASVUNIKE IDRLNEVAKNLQESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 31 Nucleic acid sequence of S-(deg-S2) for Delta S-2P strain (from 5'- end to 3'-end)	atgftcgtcttctcgtgctcgtgctcgtgctcctcctcagtgctcaatctgcgaactcggactcagctcca cctgctataactaatagtctcaccagaggcgtgtactatcctgacaagggtttagaagctccgtgctgcaactc acacaggatctgtttctccattcttagcaactgacctggttccagccatccacgtgagcggcaccaatgg cacaagcgggttcgacaatcccgctgctgcttttaacgatggcgtgtactcgcctctatcgagaagagcaac atcagaggtggtgatttggcaccacactggactccaagacacagtctctgctgacgtgaacaatgcca ccaactggtgcatcaagggtgctcgagttcagtttgaatgacccctcctggacgtgactatcacaagaaca ataagagctggatggagtcggagtgattctagcgccaacaactgcacattgagtagctgagccagccttt cctgatggacctggaggcgaagcagggaatttcaagaacctgaggagggttctgtttaagaatatcgacgg ctacttcaaatctacttaagcacacccccatcaacctggtgcgcgacctgctcagggttcagcgccctg gagccctggtggtgctgctatcgccatcaacatcaacctggttcagacactgctgcccctgcacagaagc tacctgacacccggcactcctctagcggatggaccgcccgcctgcccctactatgtgggtacctcca gcccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtgattgcgcccgtgacc ccctgagcgagacaagtgtactgaagtctttaccgtggagaaggcctatcatcagacatccaattcag ggtgcagccaaccgagctatcgtgcgtttcctaatacacaaacctgtgccatttggcgaggtgtcaacg caaccgcttcgcagcgtgtacgcctggaatagggaagcggatcagcaactgcgtggcgcgactatagcgtg

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SEQ ID NO: 32 Protein sequence of S- (deg-S2) for Delta S-2P strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSNGTKRFDNPVLPFNDGVYFASI EKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIVKVFCEQFCNDPFLDV YYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLR EFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT LLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTTT DAVDCALDPLSEKTKLSFTVEKGIYQTSNFRVQPTESIVRFPNITNL CPFGEVFNATRFASVYAWNKRISNCVADYSVLVNSASFSTFKCYG VSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLDD FTGCVIAWNSNNLDSKVGGNYNRYRLFRKSNLKPFRDISTEIYQA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAP ATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGR

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SEQ ID NO: 33 Nucleic acid sequence of S-(S2-1194) for Wuhan S-2P strain (from 5'-end to 3'-end)	atgttcgtcttcctggctctgctgctctggtctctctcagtgctgcaatctgacaactcggactcagctgcc cctgcttataactaatagcttcaccagaggcgtgtactatcctgacaagggttttagaagctccgtgctgcaact acacaggatctgtttctgccattcttagcaacgtgacctggttcacgccatccacgtgagcggcaccatgg cacaagcgggtcgacaatcccgctgctgcttttaacgatggcgtgtactctgacctacccagagaagagcaac atcatcagaggctgctgctttggcaccacactggactccaagacacagctctctgctgacgtgaacaatgcc ccaacgtggatcatcaagggtgctcgaggtccagttttgtaagtatcccttctctggcgtgtactatcacagaaca ataagagctggatggagtcgagtttagagtgtattctagcgaacaactgcacattgagtagctgagcca gcttctctgagtgacctggagggaagcagggaatttcagaacctgaggagctgtgtttaagaatctc gacggctacttcaaatctactctaaacacacccatcaacctggtgctgacgtcctcagggtctcagc gccctggagccctggtggtatctgctatcggcatcaacatcacccggttcagacactgctggccctgcac agaagctactgcaccccgccgactcctctagcggatggacggcggcgtgctgcgcctactatgtgggcta cctccagcccccagacctctctgctgaagtacaacgagaatggcaccatcacagacgcagtggaattgcgcc tggacccctgagcgagacaaagtgtacactgaagtcctttaccgtggagaagggcacatcatcagacatcca atttcagggtgcagccaaccgagctatctgtgctgcttctaatatcacaaacctgtgccattggcgagggtg teaacgcaaccgcttgcagcgtgtacgctggaatagggaagcgatcagcaactgcgtgctgacgactat agcgtgctgtacaactcgcctctttcagcacctttaagtctatggcgtgtccccacaaagctgaatgacct gtgctttaccaacgtctacgcccattcttctgtatcagggcgacgaggtgctgcccagatgcgcccgccca gacaggcaagatgcagactacaattataagctgccagacgattcacccgctgctgctgacgcttgaacag caacaatctggattccaaagtggcgccgaactacaattatctgtaccggctgtttagaagaagcaatctgaag cccttcgagagggaatctctacagaatctaccaggccggcagcacccttgcattggcgtgagggtctt aactgttatttccactccagtcctacggcttcagcccaaacggcgtgggtatcagccttaccgctggt gggtgctgagctttgagctgctgcagccccagcaacagtgtgctggcccaagaagtcaccaatctggtga agaacaagtgcgtgaactcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaacaagaa gttctgccaattcagcagttcggcaggacatcgagataccacagacgcgctgctgacccacagacc tgagatcctggacatcacacctgctcttctggcggtgagcgtgatcacaccggcaccatacaagca accagggtggcgtgctgtatcaggacgtgaattgtaccgaggtgccgtggctatccagccgatcagctga ccccaatggtgggtgtacagcacggctccaacgtcttcagacaagagccggtgctgacggagc agagcagtgacaactctatgagtgacacatccaatcggcgccgcatctgtgctcttaccagacca gacaaactctccagaagagcccgagcgtggcctccagctatcatcgctataccatgtccctggcg cgagaacagcgtggcctactctcagaatagcatgccatcccaaccagttcacatctctgtgaccacaga gatcctgccgtgtccatgaccaagacatctgtggactgcacaatgtatatctgtggcgattctaccgagtgca gaacctgctgctccagtacggcagctttgtaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggaggtgttgcggcagggtgaagcaatctacaagacccccctatcaaggacttt ggcggttccaatttccagatcctgctgatccatcaagccttctaagcggagctttatcaggacctgct gttcaacaagggtgacctggcgatgcggcttcatcaagcagtatggcgattgctggcgacatcgacgc

[illegible]

267
266

for Wuhan S-2P strain
(from 5'-end to 3'-end)

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SEQ ID NO: 36 Protein sequence of S-(deg-RBD-801) for Wuhan S-2P strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVFCEQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITR FQTLALHRSYLTGDSGSGWTAGAAAYVGYLQPRTFLLKYNENG TTDAVDCALDPLSETKCTKLSFTVEKGIYQTSNFRVQPAEAIVRFPQI TNLCPFGVEVFQATRFASVYAWNKRKISNCVADYSVLVNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQKF GRDIADTTDAVRDPQTLTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGNSVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQNSPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFQFS QILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPLLTDEMIAQYTSALLAGTTTSGWTFGAGAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDPPAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDK AHFPREGVFVSNNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFNHTSPDVLGDISGINASVUNIKE IDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVKGVKLHYT
SEQ ID NO: 37 Nucleic acid sequence of S-(deg-RBD-1194) for Wuhan S-2P strain (from 5'-end to 3'-end)	atgttcgtcttctggtctctgctgctctggtctctctcacagtgcgtcaatctgacaactcggactcagctgcca cctgcttatactaatagcttcaccagaggcgtgtactatcctgacaagggtgttagaagctcgtgctgactet acacaggatctgtttctgccattcttagcaactggaactggttccacgccatccactgagcggcaccatgg cacaagcggctcgacaatcccgtgctgcttttaacgatggcgtgtacttcgctctaccgagaagagcaac atcatcagaggctggtatcttggcaccacactggactccaagacacagtctctgctgacgtgaacaatgcca ccaacgtggtcatcaagggtgtgaggttccagtgttgaatgaccccttctggcgtgtactatcacaagaaca ataagagctggatggagtcgagtttagagtgattctagcgccaacaactgcacattgagtagctgagcca gccttctgagtggaactggagggaagcagggaattcaagaacctgagggaagtgctgttgaagaatac gacggctacttcaaaatctactctaaacacaccccatcaactggtgctgacgtgctcagggcttcagc gccttgagccctggtggtatctgctatcggtcatcaacatcacccggttcagacactgctgcccctgcac agaagctacctgacacccggcagctctctagcggtgagccggcgctgcccctactatgtgggcta cctccagccccggaccttctgctgaagtacaacgagaatggcaccatcacagacgcagtggtgctgccc tggacccctgagcagacaaaagtgtactgaagtcctttaccgtggagaaggcgtatctatcagacatcca attcaggggtgcagccagcggagcgatgctgctgcttctgaaatcacaaacctgtgccatttgaggaggt gttcgaggcaacccgcttcgagcgtgtacgcctggaataggagcggtatcagcaactgcgtggcggact atagcgtgctgtacaactcgcctcttctcagcaccttaagtgtatggcgtgtcccccacaagctgaatgac ctgtgctttaccaacgtctacgcgattcttctgtatcagggcgacgaggtgctgcccagatgcggccggc cagacaggcaagatcgagactacaattataagctgccagacgatttaccggctgctgtatcgccctggaac agcaacaatctggattccaaagtggcggaactacaattatctgtaccggctgtttagaagaagcgaatctga agcccttcgagaggacatctctacagaaatctaccaggccggcagcacccttgcaatggcgtggagggc tttaactgttattccactcagctctacggcttcagcccacaaacggcgtgggctatcagccttaccgctg gtggtgctgagctttgagctgctgacgccccagcaacagtgtgcccgaagaagtcaccaatctggtg aagaacaagtgcgtgaacttcaactcaacggcctgaccggcacaggcgtgctgaccgagtcacaagaag

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SEQ ID NO: 38 Protein sequence of S- (deg-RBD-1194) for Wuhan S-2P strain (from N-terminus to C- terminus)	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVCFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFK NLREFVFNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINTR FQTLALHRSYLTGPDSSSGWTAGAAAYVGYLQPRTFLLKYNNENG TTDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPAEIVRFPQI TNLCPFGEVVFQATRFASVYAWNKRKISNCVADYSVLVNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRIAPGQTGKIADYNYKLP DDFTGCVIAWNSNLDKSVGGNYNYLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQF GRDIADTTDAVRDPQTLTITPCSFGGVSIVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTRVYSTGSNVFQTRAGCLIGAHEVNS YECDIPIGAGICASYQTQTSNPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPPLTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFA MQMAYRFNGIGVTVQNVLYENQKLIANQFNSAIGKIQDLSSTASALG

	KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHGDK AHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKE IDRLNEVAKNLQESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAVM VTIMLCMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 39 Nucleic acid sequence of S-(deg-RBD-122- 165-234) for Wuhan S- 2P strain (from 5'-end to 3'-end)	atgttcgtcttctggtctctgctgctctggtctctctcacagtgcgtcaatctgacaactcggactcagctgcca cctgcttataactaagcttaccagaggcgtgactatcctgacaagggtttagaagctccgtgctgactct acacaggatctgtttctgcatctttagcaacgtgactggttcacgccatccacgtgagcggcaccatgg cacaagcgggttcgacaatcccgctgctgcttttaacgatggcggtgactctgctctaccgagaagagcaac atcatcagaggctggatcttggcaccacactggactccaagacacagctctgctgctgacgtgaaccaagcca ccaacgtggtcatcaaggctgctgaggttcagttttgtaagatccctcctggcgctgactatcacaagaaca ataagagctggatggagtcgagtttagagtgattctagcgccaaccagtgcacattgagtagctgagcca gcttctctgatggactggaggcgaagcagggaatttcaagaacctgaggaggtctgttttaagaatctc gacggctacttcaaaatctactaagcacaccccacacactggctgctgagctgctcagggcttcagc gcttggagccctggtggtatctgctatcggcatcagatcaccgggttcagacactgctggccctgcac agaagctactgacacccggcgactcctctagcggtgagcgccggcgctgcccactatgtggccta cctccagcccccggacttctgctgaagtacaacgagaatggcaccalcacagacgagtgagtgagcc tggacccctgagcagacaaagtgtacactgaagtccttaccgtggagaaggcgatctatcagacatcca attcagggctgagccagccgagggagctgctgcttctgaaatcacaacctgtgccatfttggcgaggt gttcgaggcaaccgcttgcagcgtgtacgctggaataggagcgatcagcaactgcgtggccgact atagcgtgctgataactcgcctcttcagcacctttagtctatggcggtgctccccacaagctgaatgac ctgtgcttacaacgtctacgcgacttctgctgacagggcgacgaggtgctgagatgccccggc cagacaggcaagatcgcagactacaattataagctgccaagcagatttaccggctgctgctgagcctggaac agcaacaatctggaatccaagtggcggaactacaattatctgtaccggctgtttagaagagcaatctga agcccttcgagaggacatctacagaaatctaccaggccggcagcacccttgaatggcgtggagggc tttaactgttatttccactcagctctacggcttcagcccacaacggcggtggcctatcagccctaccgctg gtggtgctgagctttgagctgctgcacgccccagcaacagtgtgctggcccccaagagtcaccaatctggtg aagaacaagtgcgtgaacttcaacttcaacggcctgacggcagaggcgtgctgaccgagtcacaagaag gttctgctcatttcagcagttcggcaggacatcgcagataccacagacgcgtgctgagccacagaccc tggagatcctggacatcacacctgctcttctggcggtgagcgtgacacacccggcaccatacaagca accagggtggcgtgctgtatcaggacgtgaattgtaccgaggtgcccgtggtatccacgcccagatcagctga ccccacatggcggtgtacagcaccggctccaacgtcttcagacaagagccggatgcctgacggagc agagcacgtgaacaattctatgagtgacatcacaatcggcgccggcatctgtgctcttaccagacca gacaactctccagaagagcccgagcgtggcctccagctatcatcgcctataaccatgtccctggcgcc cgagaacagcgtggcctacttaacaatagcatcgccatccaaccaacttcacaatctctgtgaccacaga gatcctgcccgtgtccatgaccaagacatctgtgactgcacaatgtatatctgtgctgagttctaccgagtgca gcaacctgctgctcagtagggcagctttgtaccagctgaatagagccctgacaggcatcgccgtggagc aggataagaacacacaggagggtgtgcccagggtgaagcaaatctacaagacccccctatcaaggacttt ggcggttcaatttttccagatcctgctgacatccaagccttcaagcggagctttatcaggacctgctg ttcaacaaggtagccctggcgatgcggcttcacagcagtagtgagctgctggcgacatcgagcc agggacctgctgctgcccagaagtttaattggcctgacgtgctgcccacctgctgacagatgagatgac gcacagtacacaagcgccctgctggccggcaccatcacatccgagtgaccttcggcgagggagccgcc tccagatccccttggcatgagatggcctataggttcaacggcatcggtgacccagaatgtgctgtacga gaaccagaagctgacgccaatcagtttaactcggcatcggcaagatccaggacagcctgtcctctacagc cagcgccctggcgaagctccagatgtgtgaatcagaacgccaggccctgaantacctggtgaagcag ctgagcagcaactcggcgccatctctagcgtgctgaatgacatcctgagccggctggacccggaggc agaggtgcagatgacccggctgacacccggcggctccagagcctccagacctatgtgacacagcagctg atcaggcccgagatcaggccagcgccaatctggcagcaaccaagatgtccgagtgctgctgctggc

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SEQ ID NO: 40 Protein sequence of S- (deg-RBD-122-165- 234) for Wuhan S-2P strain (from N-terminus to C-terminus)	MFVFLVLLPLVSSQCVNLITRTQLPPAYTNSFTRGVVYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLD SKTQSLIVNQATNVVIK VCEFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANQCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGIQITR FQTLALHRSYLT PGDSSSGW TAGAAAYVVGYLQPRTFLLKY NENG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPAEAIVRFPQI TNLCPFGEVFQATRFASVYAWN RKRISNCVADYSVL YNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYR LFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH APATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQQF GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTG SNVFQTRAGCLIGAEHVNS YECDIPIGAGICASYQTQTN SPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMITKTSVDCTMYICGDSTEC SNLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPPLLTDEMI AQYTSALLAGTITSGWTFGAGAA LQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGA ISSVLNDILSRLDPPAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH DGK AHFPREGVFVSNGTHW FVTQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASV VNIQKE IDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPV LKGVKLHYT
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SEQ ID NO: 44	LDSKVGGNYN
SEQ ID NO: 45	KSNLKPFERDIST
SEQ ID NO: 46	KPFERDISTEYQAG

SEQ ID NO: 47	GPKKSTNLVKNKC
SEQ ID NO: 48	NCDVVIGIV[N]NTVY
SEQ ID NO: 49	PELDSFKEELDKYFK[N]HTS
SEQ ID NO: 50	VNIQKEIDRLNEVA
SEQ ID NO: 51	NL[N]ESLIDLQ
SEQ ID NO: 52	LGKYEQYIKWP
SEQ ID NO: 53 Delta: SARS-CoV-2 Delta Strain Spike protein sequence	MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSGTNGTTRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSKTQSLIVNNAATNVVIKVCEFQFCNDPFLG VYYHKNNKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNL REFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQ TLLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTI TDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITN LCPFGVEFNATRFASVYAWNRKRISNCVADYSVLNSASFSTFKCYG VSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDD FTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKPFERDISTEYQA GSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAP ATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQFGR DIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTGNSVFQTRAGCLIGAHEVNNSE CDIPIGAGICASYQTQTSRARRARSVASQSIIAYTMSLGAENSVAYS NSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLQYGS FCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQIL PDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQK FNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKL QNVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDR LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF CGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNTTAPAICHGDKAH FPREGVFVSNGTHWFTVQRNFYEPQIITDNTFVSGNCDVVIGIVNNT VYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEID RLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTI MLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
SEQ ID NO: 54 WH: SARS-CoV-2 Wuhan-Hu-1 strain Spike protein sequence	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSV LHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFAS TEKSNIIRGWIFGTTLDSKTQSLIVNNAATNVVIKVCEFQFCNDPFLG VYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITR FQTLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENG TITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNI TNLCPFGVEFNATRFASVYAWNRKRISNCVADYSVLNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLP DDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEY QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLH

	APATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQF GRDIADTTDAVRDPQTLEILDITPCSFGGVSUITPGTNTSNQVAVLYQ DVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNNS YECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAY SNNSIAIPTNFTISVTTEILPVSMKTSTVDCTMYICGDSTECNLLLQY GSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFS QILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICA QKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDK AHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVN NTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKE IDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM VTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLGKVKLHYT
SEQ ID NO: 55 RBM-001	VNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVT WFHAIHVSGTNGTKRFDNPVLPFNDGVYFASIEKSNIIRGWIFGTTLT SKTQSLIVNNAATNVVIKVCDFQFCNDPFLDVYYHKNNKSWMESGV YSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSK HTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGDS GWTAGAAAYYVGYLQPRFTLLKYNENGTTITDAVDCALDPLSETKCT LKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVY AWNKRKISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYA DSFVIRGDEVQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSK VGGNYNYRYRLFRKSNLKPFERDISTEYQAGSKPCNGVEGFNCYFP LQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNK CVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEI LDITPCSFGGVSUITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAHEVNNNSYECDIPIGAGICASYQTQTN SRGSAGSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPV SMTKTSTVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNK VTADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPLLTDEMIAQ YTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE NQKLIANQFNSAIGKIQDSLSTASALGKLQNVVNQNAQALNTLVKQ LSSNFGAISSVLNDILSRDLPPEAEVQIDRLITGRLQSLQTYVTQQLIR AAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGV VFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFVTQ RNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDK YFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQE LGKYEQYIKWPLVPRGSPGSGYIPEAPRDGQAYVRKDGWVLLSTF LG
SEQ ID NO: 56 Smg protein	MKVKLLVLLCTFTATYAGTQCVNLTTRTQLPPAYTNSFTRGVYYPD KVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFN DGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNAATNVVIKVCDFQF CNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEG KQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDL IGINITRFQTLALHRSYLTGDSGWTAGAAAYYVGYLQPRFTLL

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	KYNENGTITDAVDCALDPLSETKCTLKSTVEKGIYQTSNFRVQPTES IVRFPNITNLCPFGEVFNATRFASVYAWNKRKISNCVADYSVLYNSA SFSTFKCYGVSPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIAD YNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFR DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL PFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVA VLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH VNNSYECDIPIGAGICASYQTQTNSPGSAGSVASQSIIAYTMSLGAEN SVAYSNNISAIPTNFTISVTTEILPVSMKTSVDCTMYICGDSTECSNL LLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGG FNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARD LICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTAS ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDPPEAE VQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQS KRVDFCGKGYHLMSPQSAPHGVVFLHVTYVPAQEKNTTAPAICH DGKAHFREGVVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVV NIQKEIDRLNEVAKNLNESLIDLQELGKYEQDIRSLVPRGSPGSGYIPE APRDGQAYVRKDGEWVLLSTFLGHHHHH
SEQ ID NO: 57	QCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSN VTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTT LDSKTQSLNINATNVVIKVECFQFCNDPFLGVYHKNKNSWMES EFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVFKNIDGYFK IYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGP DSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTITDAVDCALDPLSE TKCTLKSTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRF ASVYAWNKRKISNCVADYSVLYNSASFSTFKCYGVSPTKLNLCFT NVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSN NLDSKVGGNYNYLYRLFRKSNLKPFRDISTEIYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTN LVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRD PQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICAS YQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNISAIPTNFTISV TTEILPVSMKTSVDCTMYICGDSTECSNLLQYGSFCTQLNRALTGI AVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIE DLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLT DEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVT QNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQAL NTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTY VTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSP QSAPHGVVFLHVTYVPAQEKNTTAPAICHDGKAHFREGVVFVSN THWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDS FKEELD KYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQ ELGKYEQY

SEQ ID NO: 58 E1	SSANNCTFEYVSQ
SEQ ID NO: 59 E2	TESIVRFPNITNL
SEQ ID NO: 60 E3	KPFERDISTEIQAG
SEQ ID NO: 61 E4	GPKKSTNLVKNKC
SEQ ID NO: 62 E5	TEVPVAIHADQ
SEQ ID NO: 63 E6	RVYSTGSNVFQTR
SEQ ID NO: 64 E7	RRARSVASQS
SEQ ID NO: 65 E8	DPSKPSKRSF
SEQ ID NO: 66 E9	FIKQYGDCLGDI
SEQ ID NO: 67 E10	ENQKLIANQFNS
SEQ ID NO: 68 E11	GKIQDSLSTA
SEQ ID NO: 69 E12	NCDVVIGIVNNTVY
SEQ ID NO: 70 E13	PELDSFKEELDKYFKNHTS

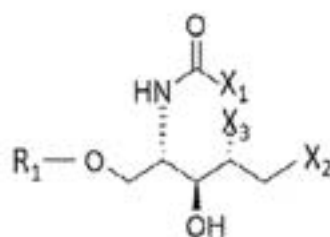
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SEQ ID NO: 71 E14	TSPDVDLGDISGINA
SEQ ID NO: 72 E15	VNIQKEIDRLNEVA
SEQ ID NO: 73 E16	NLNESLIDLQ
SEQ ID NO: 74 E17	LGKYEQYIKWP

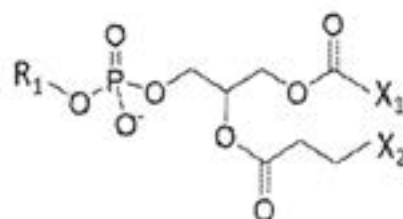
What is claimed is:

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

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



Formula 1; or



Formula 2;

wherein R_1 comprises a 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;

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

2. The pharmaceutical formulation of claim 1, wherein the spike protein comprises an amino acid substitution of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T),

wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue.

3. The pharmaceutical formulation of claim 1, wherein the spike protein comprises an amino acid deletion or addition at N-linked glycosylation sequons (N-X-S/T) to eliminate N-linked glycan sequons.
4. The pharmaceutical formulation of claim 1, wherein the spike protein comprises an amino acid substitution of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites.
5. The pharmaceutical formulation of claim 1, wherein
the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 2, 16, 18, or 20, or
an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or
80% identity to the amino acid sequence set forth in SEQ ID NO: 2, 16, 18 or 20; or
the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in
SEQ ID NO: 1, 15, 17, or 19 respectively, or a nucleotide sequence having at least about
99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set
forth in SEQ ID NO: 1, 15, 17 or 19 respectively.
6. The pharmaceutical formulation of claim 1, wherein
the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, 22, 24, or 26, or
an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or
80% identity to the amino acid sequence of SEQ ID NO: 4, 22, 24, or 26, and wherein the
spike protein comprises a receptor binding domain (RBD) lacking at least one
glycosylation sites; or
the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in
SEQ ID NO: 3, 21, 23, or 25 respectively, or a nucleotide sequence having at least about
99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence set
forth in SEQ ID NO: 3, 21, 23, or 25 respectively.
7. The pharmaceutical formulation of claim 1, wherein

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

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

8. The pharmaceutical formulation of claim 1, wherein

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

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

9. The pharmaceutical formulation of claim 1, wherein

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

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

10. The pharmaceutical formulation of claim 1, wherein

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

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

11. The pharmaceutical formulation of claim 1, wherein

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

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

12. The pharmaceutical formulation of claim 1, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44), KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46), GPKKSTNLVKNKC (SEQ ID NO: 47), NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.

13. The pharmaceutical formulation of claim 1, wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 53, 54, 55, 56, or 57, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 53, 54, 55, 56, or 57.
14. The pharmaceutical formulation of claim 13, wherein the payload encodes an immunogenic peptide comprising an amino acid sequence selected from a group consisting of:

SEQ ID NO: 58 - SSANNCTFEYVSQ;

SEQ ID NO: 59 - TESIVRFPNITNL;

SEQ ID NO: 60 - KPFERDISTEYQAG;

SEQ ID NO: 61 - GPKKSTNLVKNKC;

SEQ ID NO: 62 - TEVPVAIHADQ;

SEQ ID NO: 63 - RVYSTGSNVFQTR;

SEQ ID NO: 64 - RRARSVASQS;

SEQ ID NO: 65 - DPSKPSKRSF;

SEQ ID NO: 66 - FIKQYGDCLGDI;

SEQ ID NO: 67 - ENQKLIANQFNS;

SEQ ID NO: 68 - GKIQDSLSTA;

SEQ ID NO: 69 - NCDVVIGIVNNTVY;

SEQ ID NO: 70 - PELDSFKEELDKYFKNHTS;

SEQ ID NO: 71 - TSPDVDLGDISGINA;

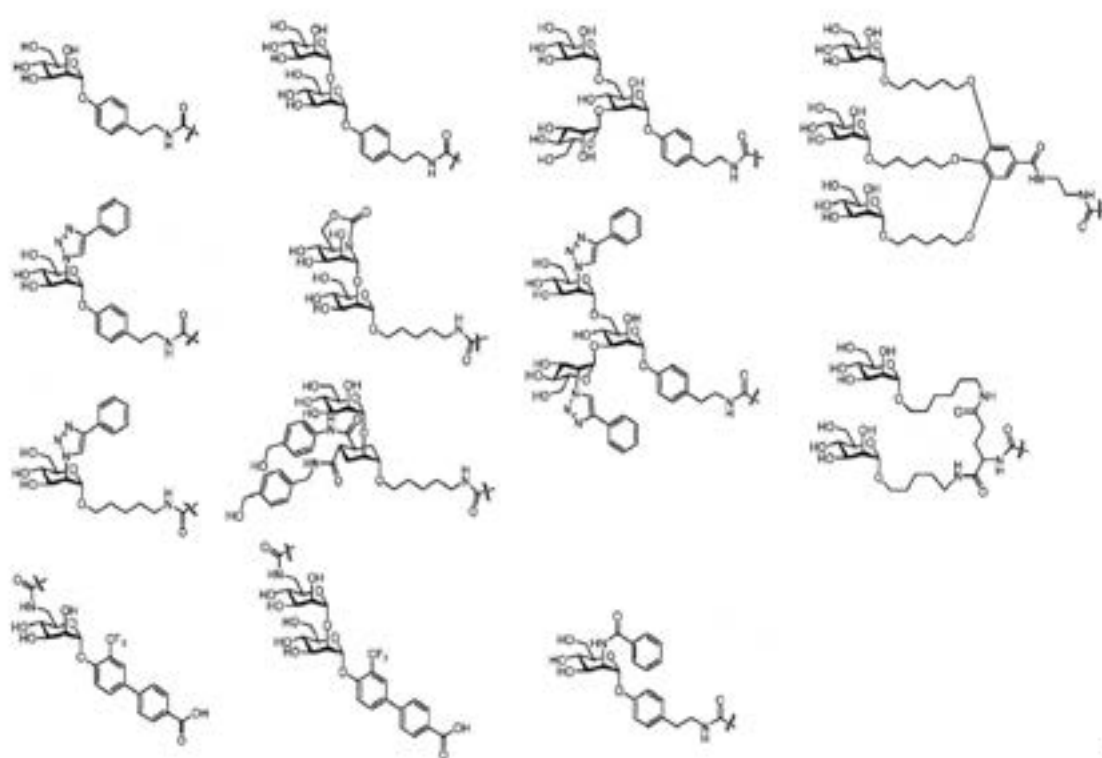
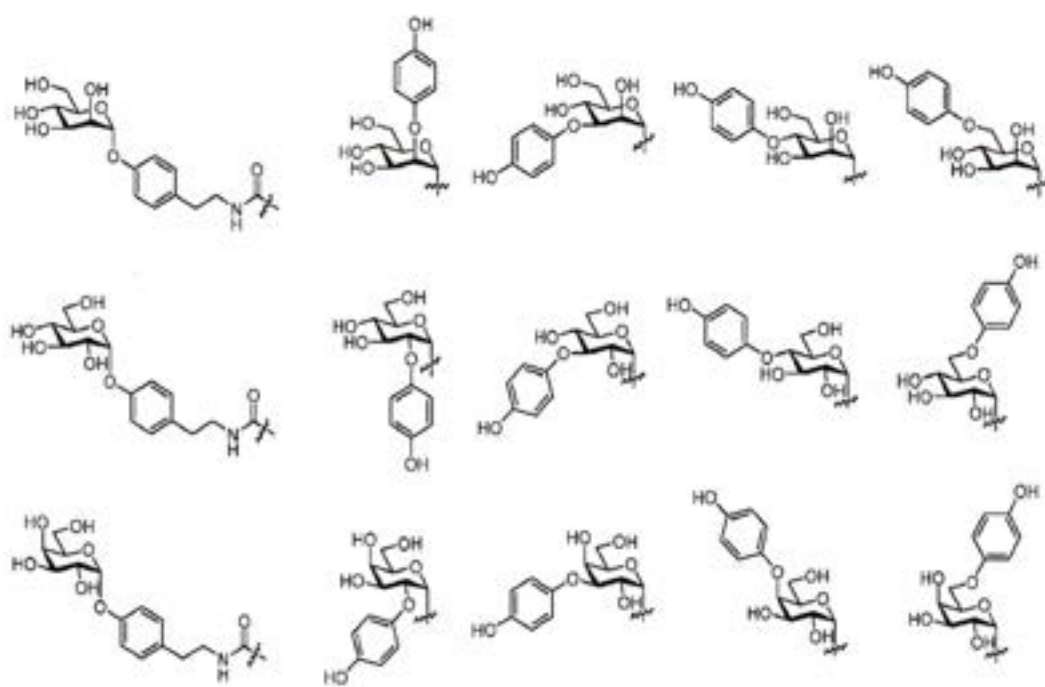
SEQ ID NO: 72 - VNIQKEIDRLNEVA;

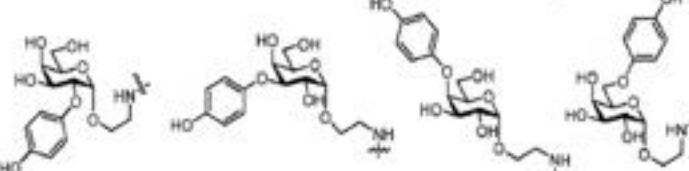
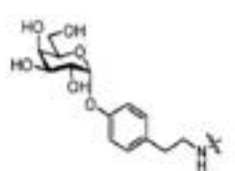
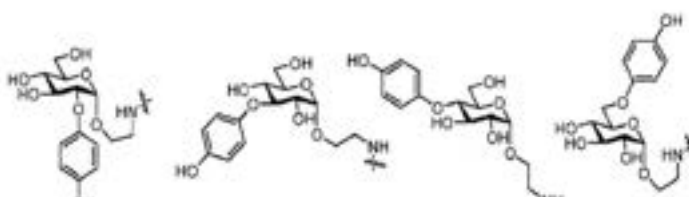
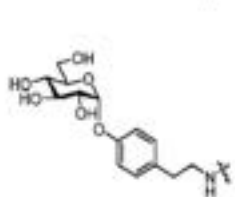
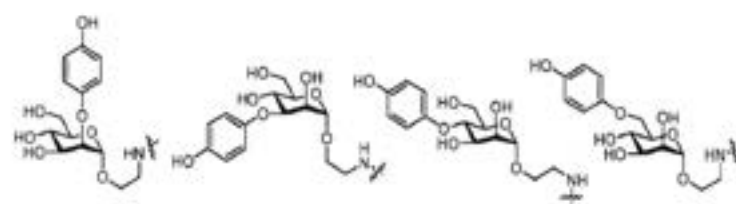
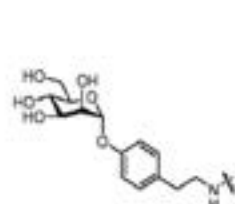
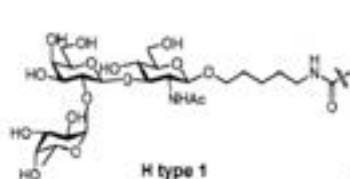
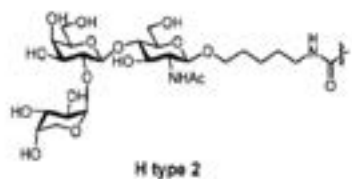
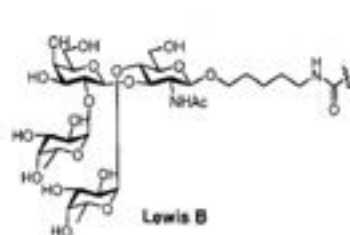
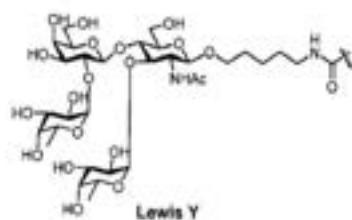
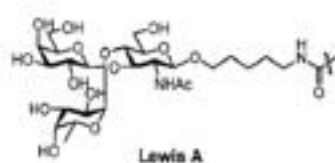
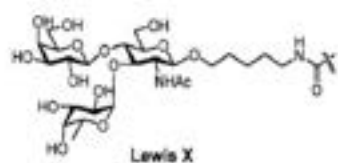
SEQ ID NO: 73 - NLNESLIDLQ; and

SEQ ID NO: 74 - LGKYEQYIKWP;

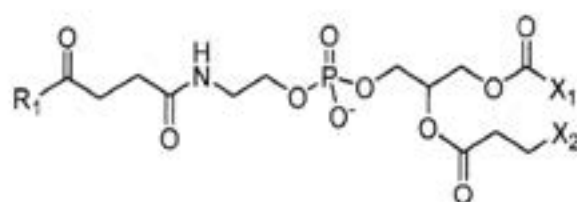
or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 58 to 74

15. The pharmaceutical formulation of claim 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.
16. The pharmaceutical formulation of claim 15, 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.
17. The pharmaceutical formulation of claim 16, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.
18. The pharmaceutical formulation of claim 1, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.
19. The pharmaceutical formulation of claim 1, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
20. The pharmaceutical formulation of claim 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.
21. The pharmaceutical formulation of claim 1, wherein R_1 is selected from the group consisting of:

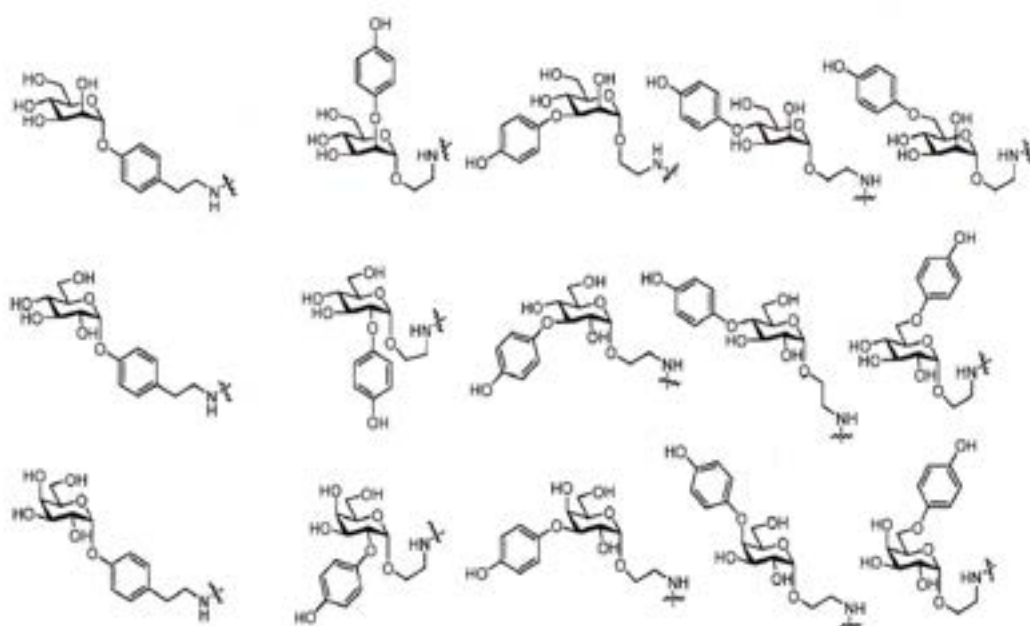




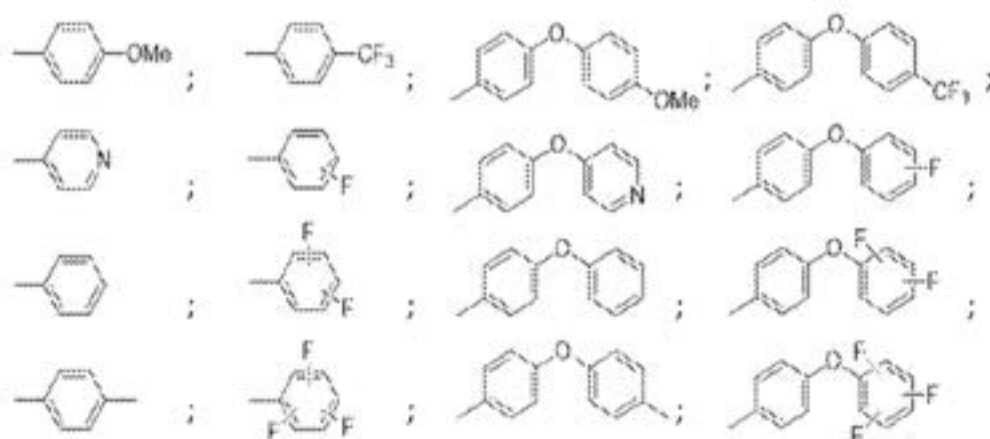
22. The pharmaceutical formulation of claim 1, wherein the compound is of Formula 3:



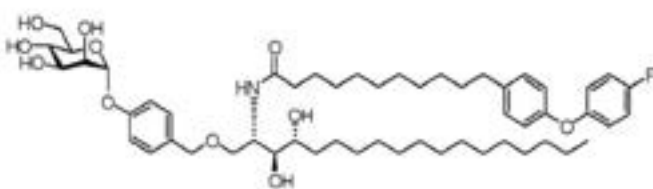
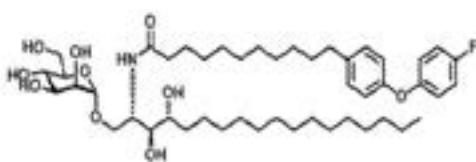
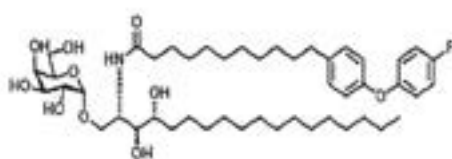
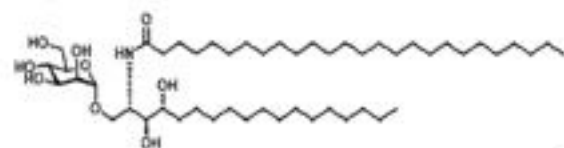
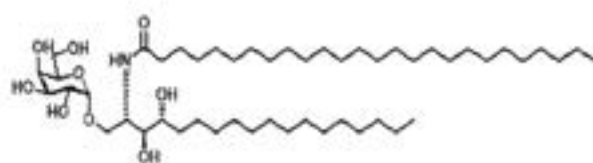
wherein R_1 is selected from the group consisting of:

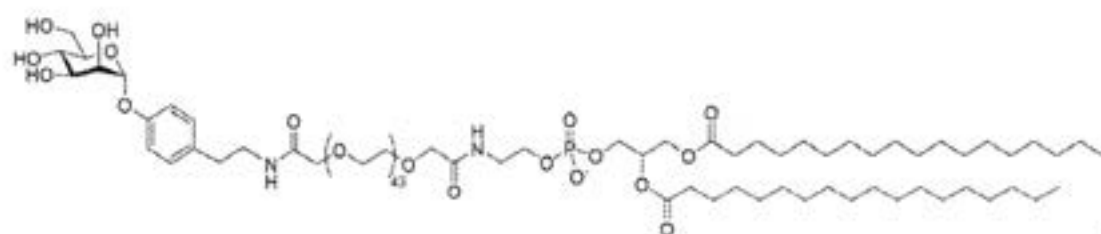
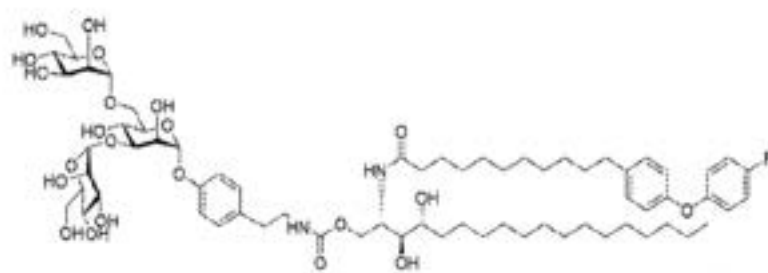
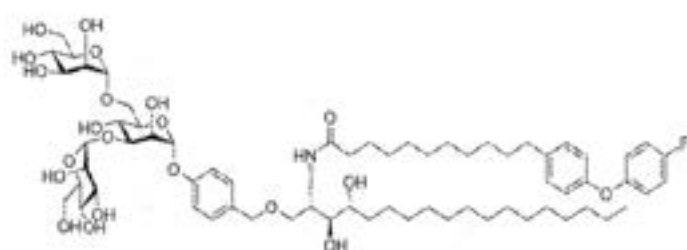
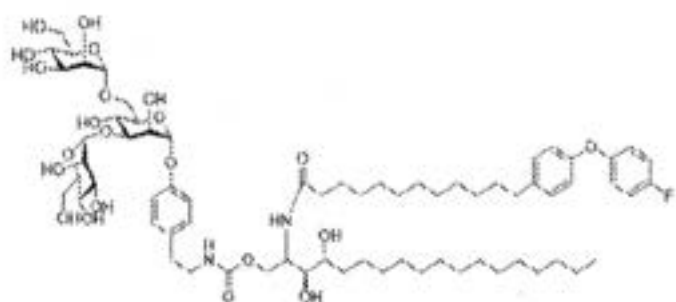
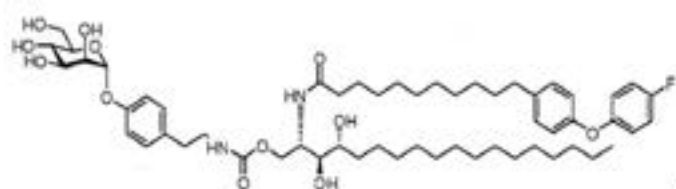
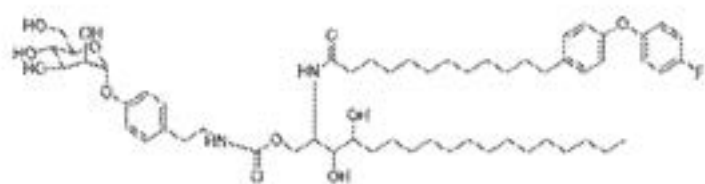


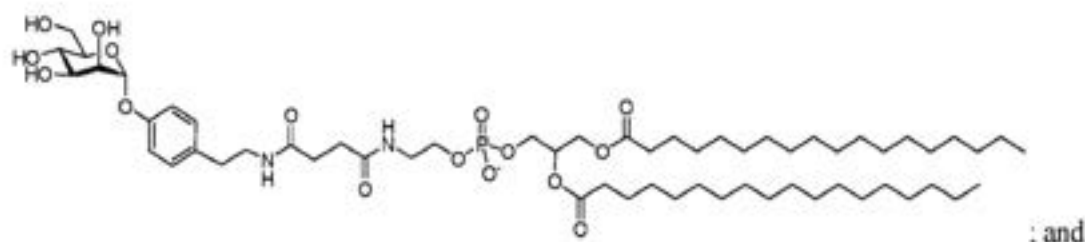
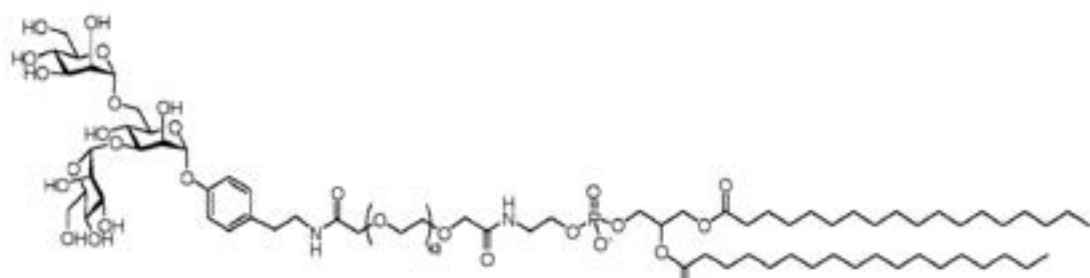
23. The pharmaceutical formulation of claim 1, wherein at least one of X₁ and X₂ comprises a saturated hydrocarbon chain, comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons.
24. The pharmaceutical formulation of claim 1, wherein X₁ and X₂ are each independently hydrogen, C₄₋₃₀ alkyl, C₄₋₃₀ alkenyl, C₄₋₃₀ alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)_nX_4$, n is 4 to 30, and X₄ is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when X₄ 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.
25. The pharmaceutical formulation of claim 1, wherein X₄ is an aryl, aryloxy, heterocyclic group, cycloalkyl, heterocycloalkyl, or a combination thereof, and wherein X₄ comprises 0 to 6 substituents, selected from the group consisting of C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halogen, and C₁₋₆ alkoxy.
26. The pharmaceutical formulation of claim 25, wherein X₄ is -R₃-O-R₄, wherein R₃ and R₄ are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halogen, and C₁₋₆ alkoxy.
27. The pharmaceutical formulation of claim 1, wherein X₄ is selected from the group consisting of:



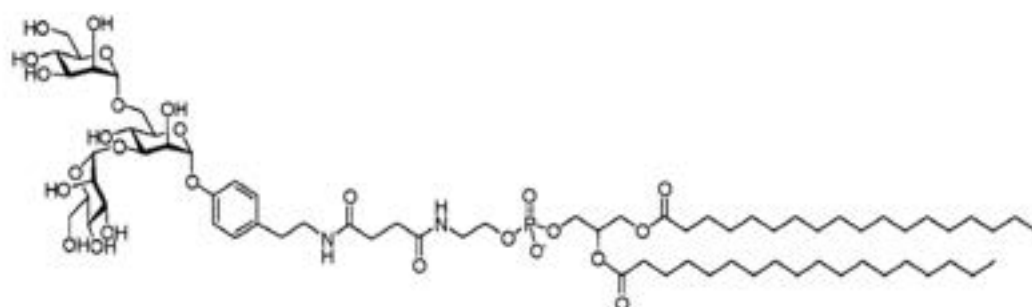
28. The pharmaceutical formulation of any one of claims 1 to 27, wherein the compound is selected from the group consisting of:







; and



29. The pharmaceutical formulation of claim 1, wherein the component is not glycolipid C34 or α -galactosylceramide.
30. The pharmaceutical formulation of claim 1, 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.
31. The pharmaceutical formulation of claim 1, further comprising an adjuvant, wherein the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, α -galactosylceramide, Aluminum salt, Squalene, MF59, or QS-21. Other examples of adjuvants in some vaccines that can be used in the composition of the present disclosure are aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), mixed aluminum salts, Freund's complete adjuvant, Freund's

incomplete adjuvant, AS03 (GlaxoSmithKline), MF59 (Seqirus), and CpG 1018 (Dynavax), or a combination thereof.

1/10

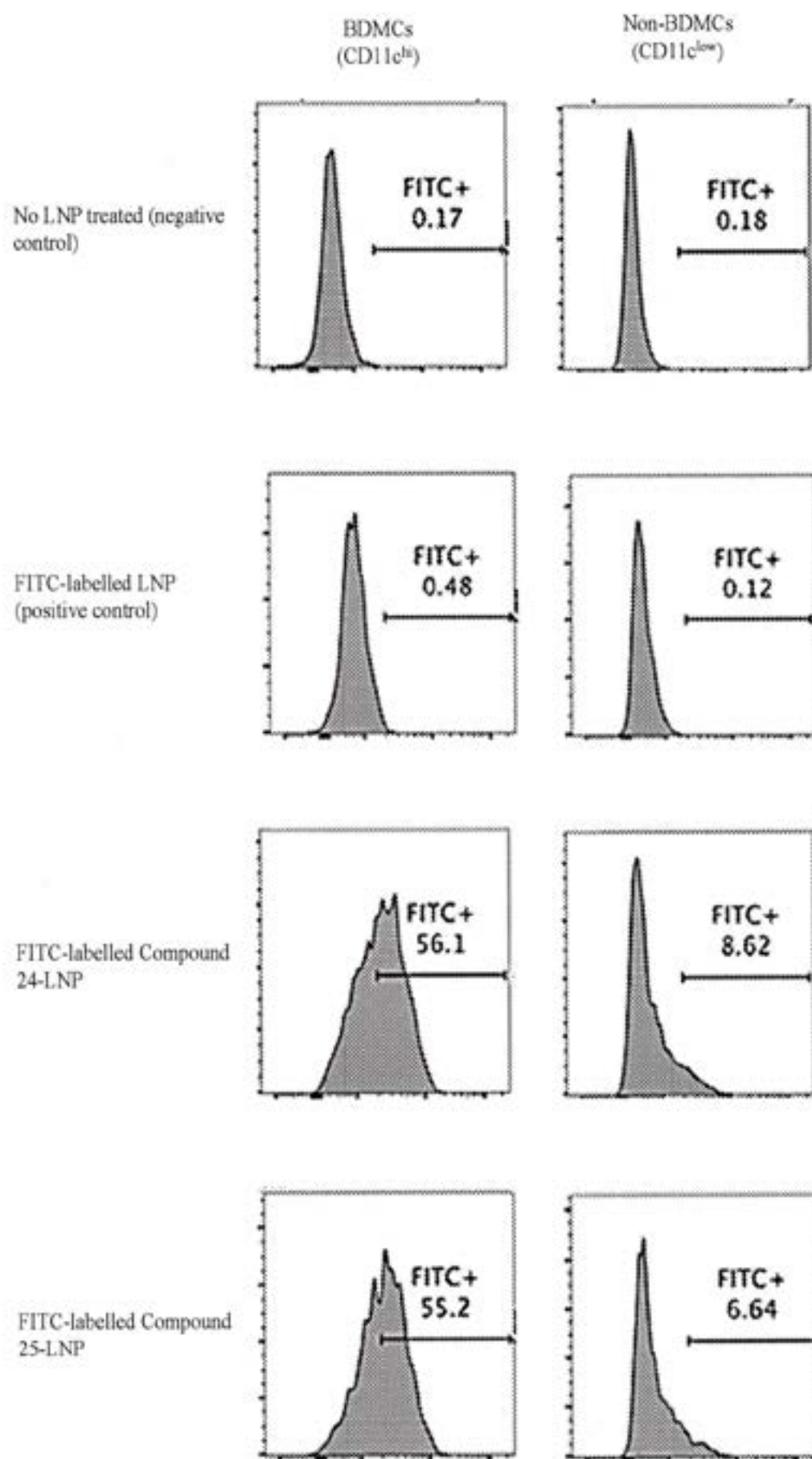


FIG. 1

SUBSTITUTE SHEET (RULE 26)

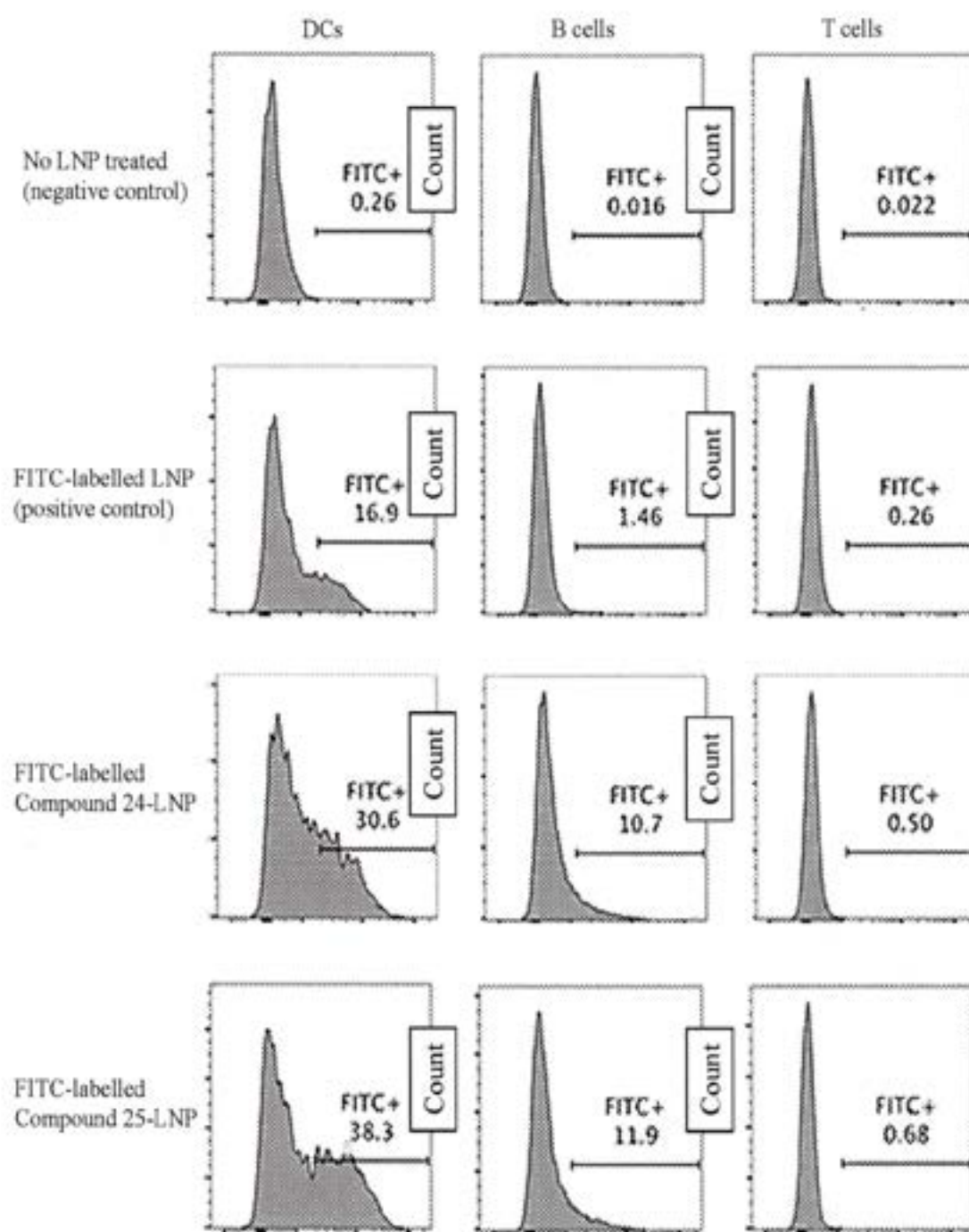


FIG. 2

SUBSTITUTE SHEET (RULE 26)

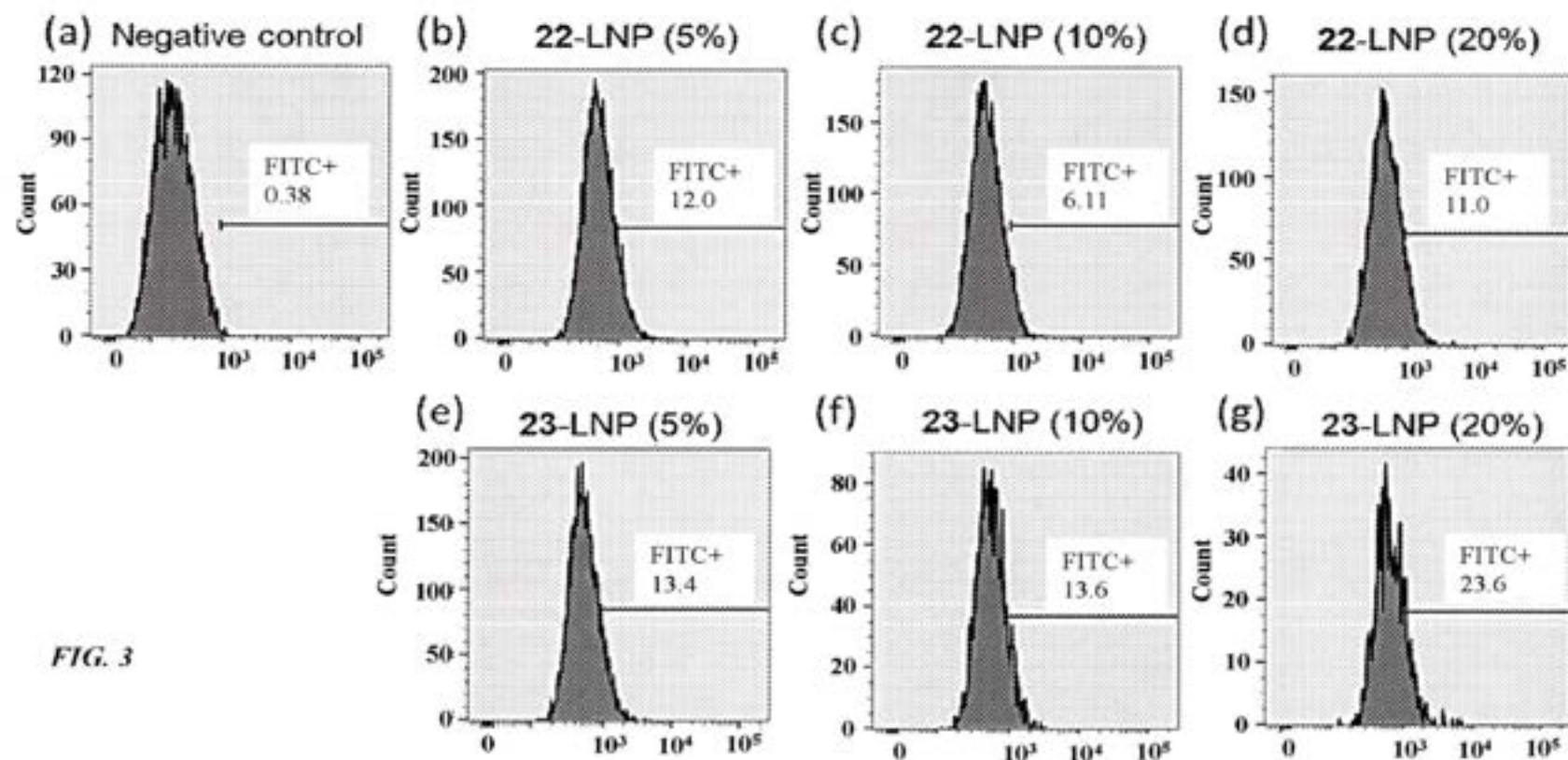


FIG. 3

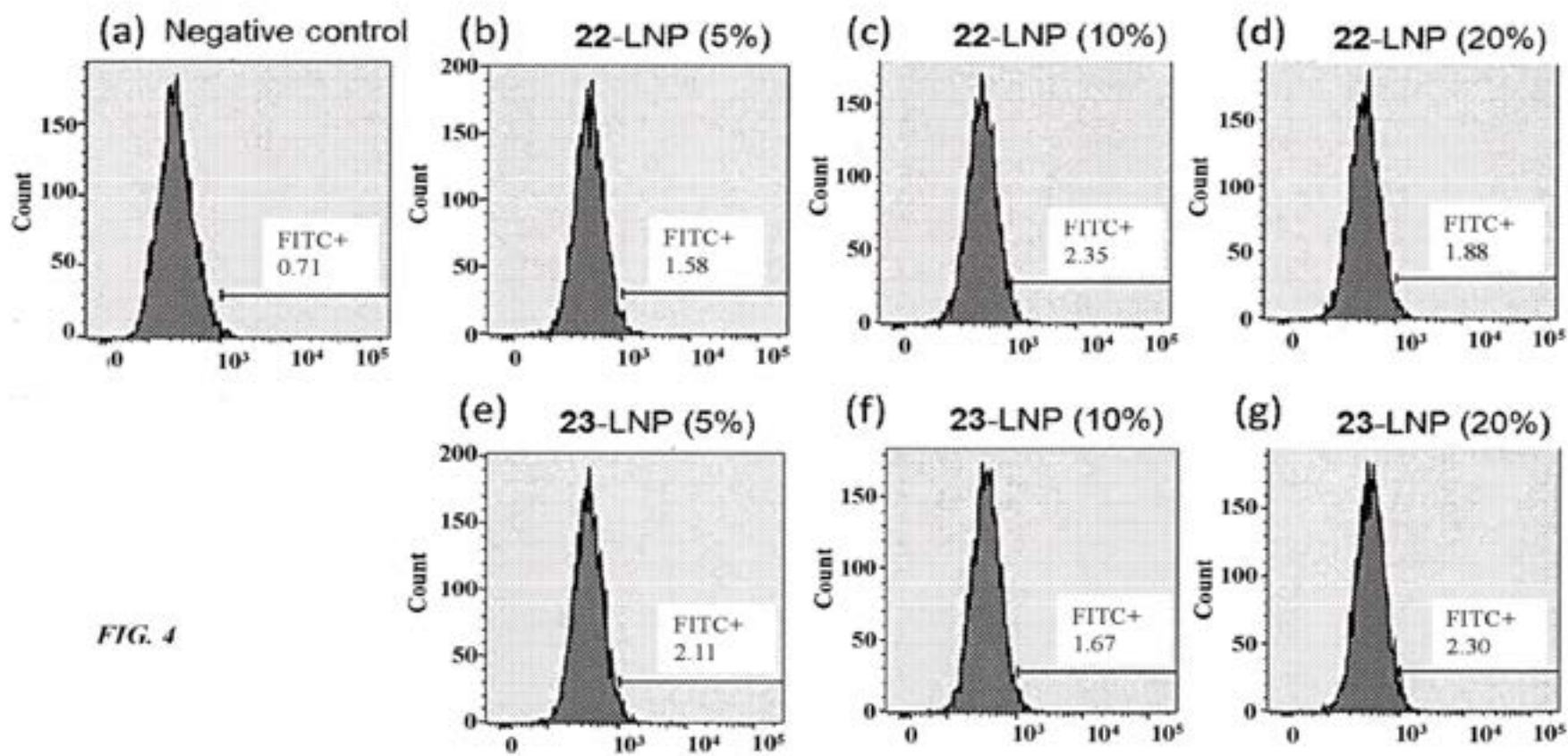


FIG. 4

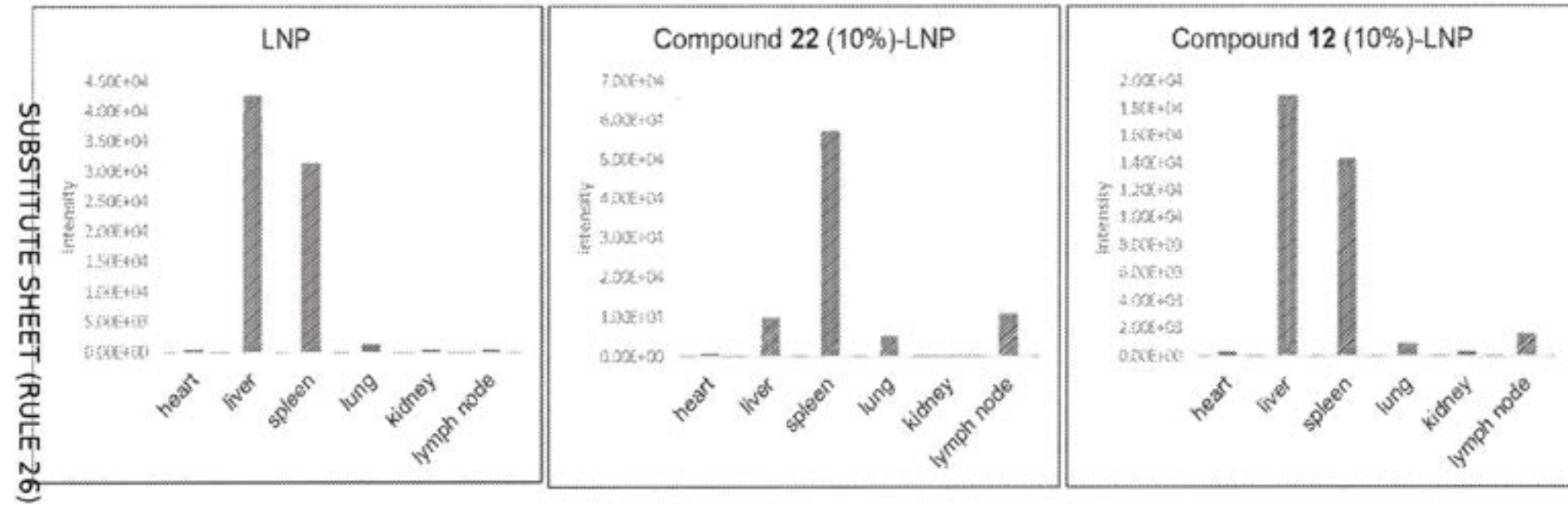


FIG. 5

SUBSTITUTE SHEET (RULE 26)

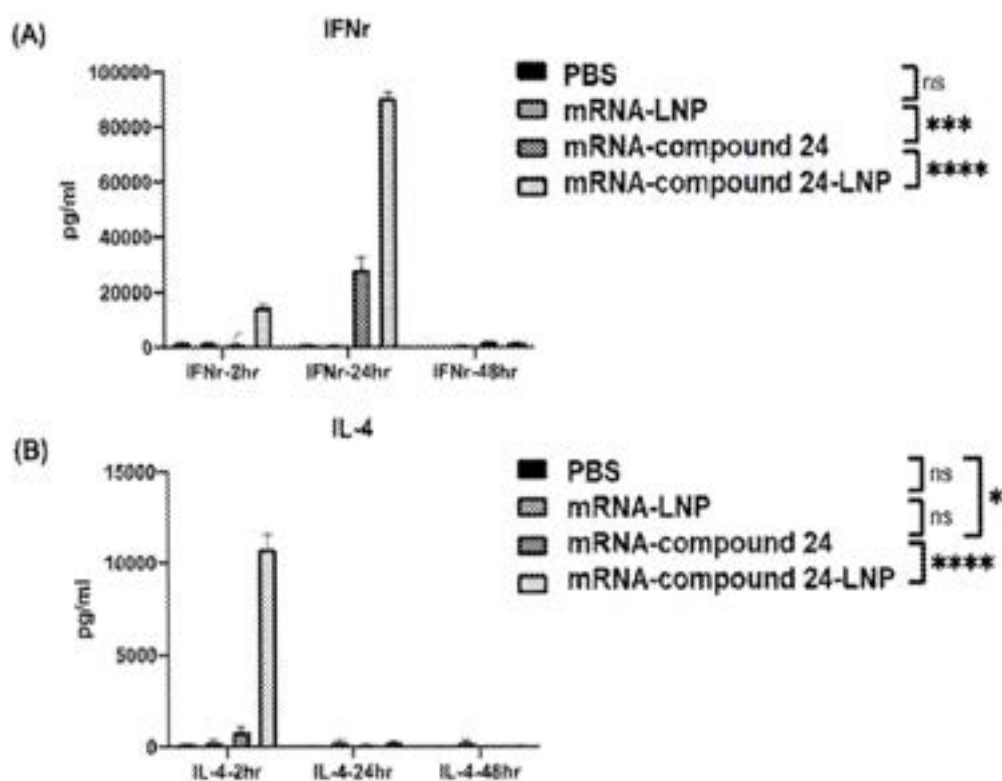


FIG. 8

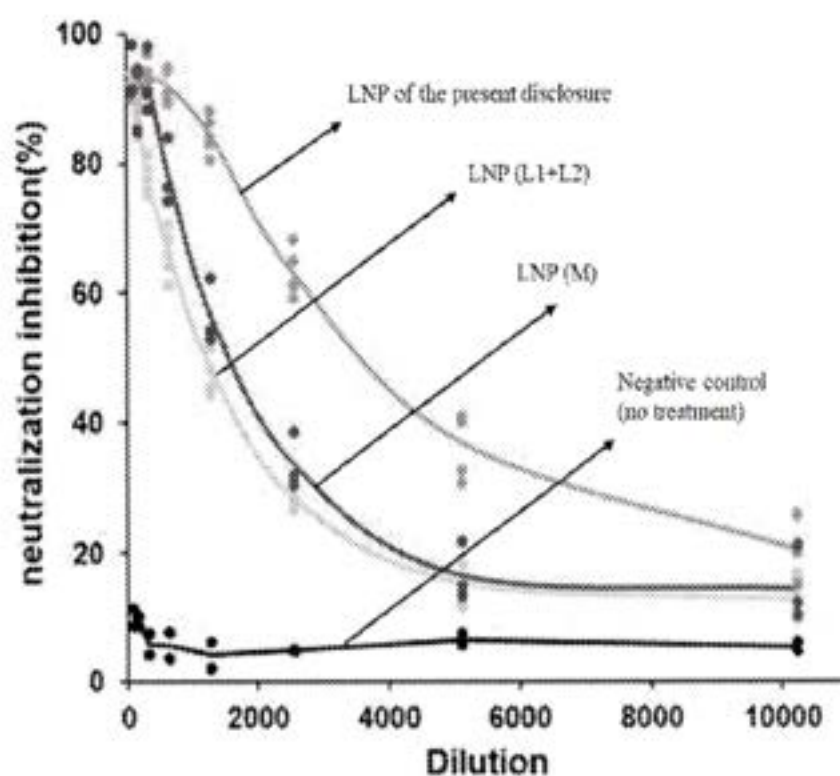


FIG. 9

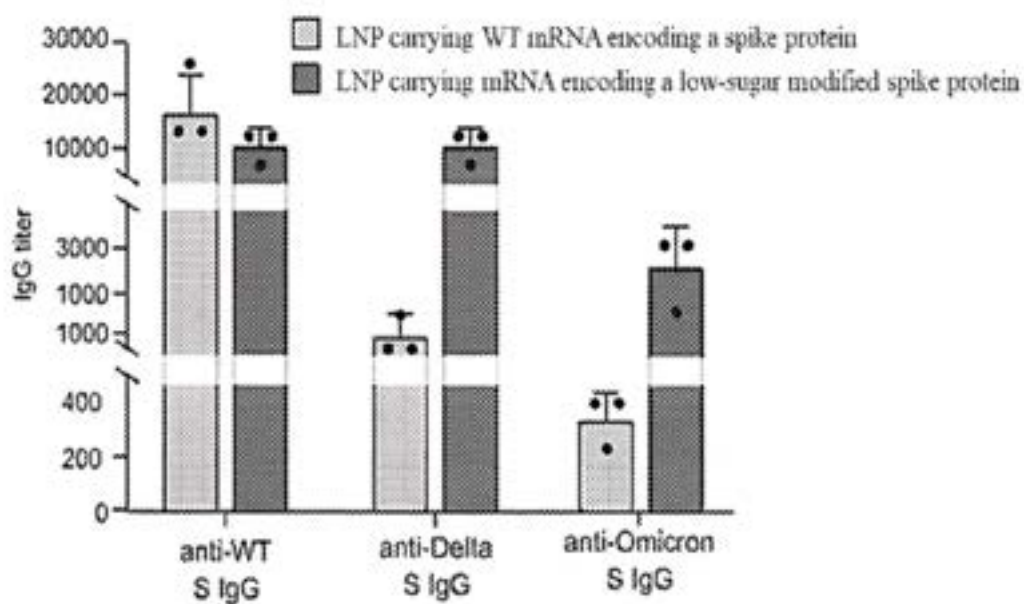


FIG. 10

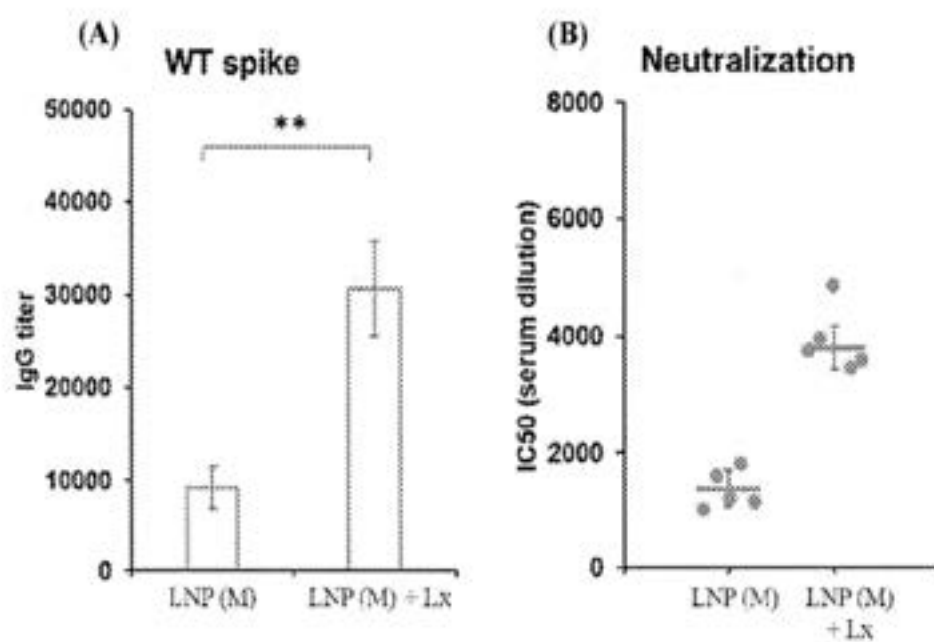


FIG. 11

SUBSTITUTE SHEET (RULE 26)

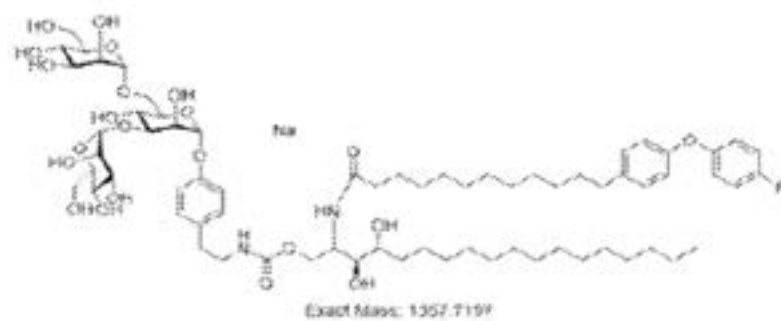
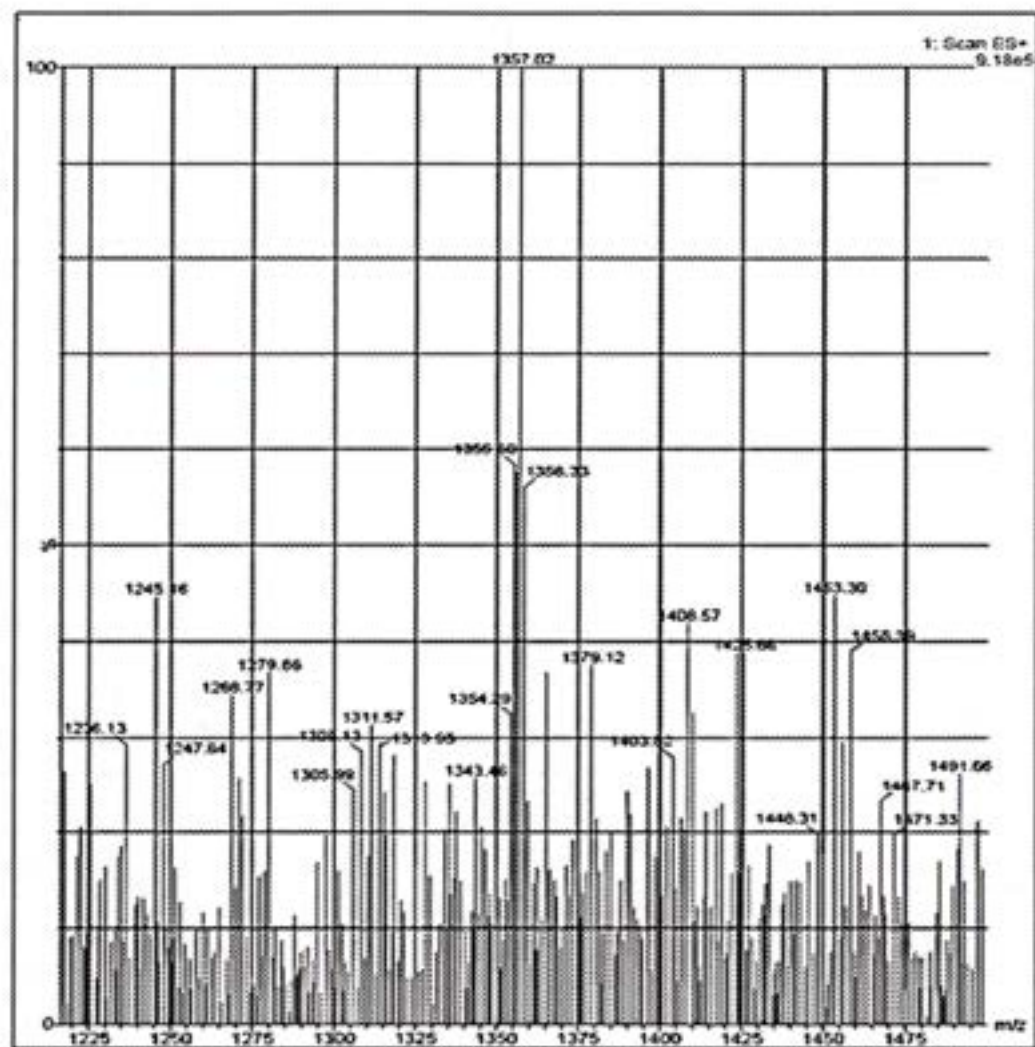


FIG. 12



PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To: WADE LIN
PROSYLA GROUP PC
530 TECHNOLOGY DR., SUITE 100
IRVINE, CA 92618
UNITED STATES OF AMERICA

Date of mailing
(day/month/year)

SEP 17 2024

Applicant's or agent's file reference

A1000-01100PCT-3

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.

PCT/US2024/023597

International filing date
(day/month/year)

08 April 2024 (08.04.2024)

Applicant Rock BioMedical Inc.

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau preferably through ePCT, or on paper to:
The International Bureau of WIPO, 34, chemin des Colombettes, 1211 Geneva 20, Switzerland

For more detailed instructions, see the *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
 - ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Within 22 months from the priority date, the applicant may request that a supplementary international search be carried out by a different International Searching Authority that offers this service (Rule 45bis.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/720 (revised January 2020)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference A1000-01100PCT-3		FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2024/023597	International filing date (day/month/year) 08 April 2024 (08.04.2024)	(Earliest) Priority Date (day/month/year) 08 April 2023 (08.04.2023)
Applicant Rock BioMedical Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 10

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/023597

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed.
- b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter I(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/023597

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***Please See Supplemental Page-***

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31;
2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31;
3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31;
4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/023597

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 39/215; A61K 9/51; A61P 31/14; C07K 14/165 (2024.01)

ADD.

CPC - INV. A61K 39/215; A61K 9/5123; A61P 31/14; C07K 14/165

ADD. A61K 2039/53; C12N 2770/20022; C12N 2770/20034

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y — A	US 2020/0078452 A1 (ACADEMIA SINICA) 12 March 2020; paragraphs [0180], [0184], [0185], [0186], [0192], [0202]	1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 --- 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31
Y — A	WO 2022/221835 A2 (ACADEMIA SINICA) 20 October 2022; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35	1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 --- 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31
Y ✓	(ALAM, MM et al.) Glycan-Modified Virus-Like Particles Evoke T Helper Type 1-Like Immune	1st invention: 18-19,

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

27 August 2024 (27.08.2024)

Date of mailing of the international search report

SEP 17 2024

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

E-mail: isa.us@uspto.gov

Authorized officer

Shane Thomas

Telephone No. PCT Helodesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/023597

305
304

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
— A	Responses. ACS Nano. Epub 17 August 2020, Vol. 15, No. 1; pages 1-29; abstract; fig. 1b; DOI: 10.1021/acsnano.0c03023	28/18-19 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 — 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31
P,Y	(FAN, CY et al.) Synthesis of Dendritic Cell-Targeted Polymeric Nanoparticles for Selective Delivery of mRNA Vaccines to Elicit Enhanced Immune Responses. bioRxiv, Epub: 14 November 2023; pages 1-12; entire document; DOI: 10.1101/2023.11.13.566827;	1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31

Continued From Box No. III: Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-31, and Formula 1 (bi-functional compound structure), C1-30 alkyl (X1, X2 in Formula 1), amino acid substitution (spike protein modification), SEQ ID NO: 4 (spike protein amino acid sequence), SEQ ID NO: 3 (spike protein-encoding RNA sequence), mono-mannoside (glycosyl group), claim 28, 1st structure (compound structure) are directed towards pharmaceutical formulations and LNPs and payloads associated therewith.

The pharmaceutical formulations, LNPs, and payloads of Claims 1 (in-part), 2, 4, 6 (in-part), 18-19 (each in-part), 23, 24 (in-part), 28 (in-part), 29-31 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass Formula 1 (first exemplary bi-functional compound structure), C1-30 alkyl (exemplary X1, X2 in Formula 1 based on first exemplary compound structure), amino acid substitution (first exemplary spike protein modification), SEQ ID NO: 4 (first exemplary modified spike protein amino acid sequence), SEQ ID NO: 3 (first exemplary modified spike protein-encoding RNA sequence), mono-mannoside (first exemplary glycosyl group), claim 28, 1st structure (first exemplary compound structure). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 1, 6, 18-19, 24, 28 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s) to be searched. Additional bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Exemplary elections would be Formula 1 (bi-functional compound structure), hydrogen (X1, X2 in Formula 1), amino acid substitution (spike protein modification), SEQ ID NO: 6 (spike protein amino acid sequence), SEQ ID NO: 5 (spike protein-encoding RNA sequence), fucoside (glycosyl group).

Groups I+ share the technical features including: a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound; wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile. These shared technical features are previously disclosed by WO 2022/221835 A2 to ACADEMIA SINICA (hereinafter "Academia").

Academia discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound; wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35).

Since none of the special technical features of the Groups I+ inventions are found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Academia reference, unity of invention is lacking.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bix.1)

To: WADE LIN
PROSYLA GROUP PC
530 TECHNOLOGY DR., SUITE 100
IRVINE, CA 92618
UNITED STATES OF AMERICADate of mailing
(day/month/year)

SEP 17 2024

Applicant's or agent's file reference
A1000-01100PCT-3

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2024/023597

International filing date (day/month/year)

08 April 2024 (08.04.2024)

Priority date (day/month/year)

08 April 2023 (08.04.2023)

International Patent Classification (IPC) or both national classification and IPC

IPC - INV. A61K 39/215; A61K 9/51; A61P 31/14; C07K 14/165 (2024.01)

ADD.

CPC - INV. A61K 39/215; A61K 9/5123; A61P 31/14; C07K 14/165

ADD. A61K 2039/53; C12N 2770/20022; C12N 2770/20034

Applicant Rock BioMedical Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bix.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion

27 August 2024 (27.08.2024)

Authorized officer

Shane Thomas

PCT Help Desk
Telephone No. 571-272-4300

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Box No. 1 Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(b)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed.
b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.

5. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 3, 5, 7-14, 16-17, 20, 22, 27

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 3, 5, 7-14, 16-17, 20, 22, 27

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing complying with WIPO Standard ST.26, and such listing was not available to the International Searching Authority in the form, language and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a).

☐ See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☒ paid additional fees.
 - ☐ paid additional fees under protest and, where applicable, the protest fee.
 - ☐ paid additional fees under protest but the applicable protest fee was not paid.
 - ☐ not paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with.
 - ☒ not complied with for the following reasons:

.-***-Please See Supplemental Page-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. .-***-Please See Supplemental Page-***-

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	***-Please See Below-***	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	***-Please See Below-***	YES
	Claims	***-Please See Below-***	NO
Industrial applicability (IA)	Claims	***-Please See Below-***	YES
	Claims	NONE	NO

2. Citations and explanations:

-Continued from Box No. V. 1: Statement:

Novelty (N): YES: Claims 1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31

Inventive Step (IS): YES: Claims 1st invention: NONE // 2nd invention: NONE // 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31

Inventive Step (IS): NO: Claims 1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 3rd invention: NONE // 4th invention: NONE

Industrial Applicability (IA): YES: Claims 1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31 // 2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 // 4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31

-Continued from Box No. V. 2: Citations and Explanations:

1st invention

Claims 1-2, 4, 6, 23-24, 28-31 lack an inventive step under PCT Article 33(3) as being obvious over US 2020/0078452 A1 to ACADEMIA SINICA (hereinafter "Academia'452") in view of WO 2022/221835 A2 to ACADEMIA SINICA (hereinafter "Academia'835").

As per claim 1, Academia'452 discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising: Formula I, wherein R1 comprises a substituted or non-substituted glycosyl group; wherein X1 and X2 are each independently C1-30 alkyl, wherein X3 is hydrogen, C1-6 alkyl, or hydroxyl; wherein the lipid nanoparticle encapsulates a payload (pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutically acceptable excipient, wherein the lipid is an alpha-GalCer having the structure identical to Formula I of the instant PCT application, wherein R1 comprises a substituted or non-substituted glycosyl group; wherein X1 and X2 are each independently C1-30 alkyl, wherein X3 is hydroxyl; paragraphs [0180], [0184], C1-first structure, [0185], [0202]). Academia'452 does not disclose a payload encoding a spike protein of Severe Acute Respiratory Syndrome Coronavirus or SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile. Academia'835 discloses a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 2, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue. Academia'835 discloses wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue (a modified nucleic acid molecule encoding a modified spike protein comprising one or more amino acid substitutions of asparagine (N) to

-Continued in Supplemental Box-

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 28 of the 3rd invention is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 28 of the 3rd invention is indefinite for the following reason(s): The additional structure #2 in the LOU response, indicated to be the 8th compound of claim 28, is absent from claim 28. A derivative structure, compound 21, is present in claim 28 and is the actual 8th structure of claim 28. Therefore, for examination purposes, the additional structure #2 of the 3rd invention is considered to be compound 21, which is the 8th structure of claim 28.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Continued From Box IV (ii): Lack of unity of invention

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-31, and Formula 1 (bi-functional compound structure), C1-30 alkyl (X1, X2 in Formula 1), amino acid substitution (spike protein modification), SEQ ID NO: 4 (spike protein amino acid sequence), SEQ ID NO: 3 (spike protein-encoding RNA sequence), mono-mannoside (glycosyl group), claim 28, 1st structure (compound structure) are directed towards pharmaceutical formulations and LNPs and payloads associated therewith.

The pharmaceutical formulations, LNPs, and payloads of Claims 1 (in-part), 2, 4, 6 (in-part), 18-19 (each in-part), 23, 24 (in-part), 28 (in-part), 29-31 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass Formula 1 (first exemplary bi-functional compound structure), C1-30 alkyl (exemplary X1, X2 in Formula 1 based on first exemplary compound structure), amino acid substitution (first exemplary spike protein modification), SEQ ID NO: 4 (first exemplary modified spike protein amino acid sequence), SEQ ID NO: 3 (first exemplary modified spike protein-encoding RNA sequence), mono-mannoside (first exemplary glycosyl group), claim 28, 1st structure (first exemplary compound structure). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 1, 6, 18-19, 24, 28 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s) to be searched. Additional bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected bi-functional compound structure(s), X1, X2 group(s) in Formula 1, spike protein modification(s), modified spike protein amino acid sequence(s), modified spike protein-encoding RNA sequence(s), glycosyl group(s), compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Exemplary elections would be Formula 1 (bi-functional compound structure), hydrogen (X1, X2 in Formula 1), amino acid substitution (spike protein modification), SEQ ID NO: 6 (spike protein amino acid sequence), SEQ ID NO: 5 (spike protein-encoding RNA sequence), fucose (glycosyl group).

Groups I+ share the technical features including: a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound; wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile. These shared technical features are previously disclosed by WO 2022/221835 A2 to ACADEMIA SINICA (hereinafter "Academia").

Academia discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound; wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35).

Since none of the special technical features of the Groups I+ inventions are found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Academia reference, unity of invention is lacking.

Continued From Box IV (iii)

1st invention: 1-2, 4, 6, 18-19, 23-24, 28-31;
2nd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31;
3rd invention: 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31;
4th invention: 1-2, 4, 6, 15, 18-19, 21, 23-31

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Continued From Box V. 2: Citations and Explanations

glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue; claims 1, 5). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 4, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites. Academia'835 discloses wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites (the spike protein comprises one or more amino acid substitutions of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites; claim 1, 3, 5). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 6, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 3. Academia'835 discloses wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites (wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 4 that has 100% identity with SEQ ID NO: 4 of the instant PCT application, wherein the modified spike protein comprises a RBD lacking glycosylation sites; claim 1, 8). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a well-defined sequence and a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 23, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein at least one of X1 and X2 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 (the glycolipid comprises the R group (CH₂)₂₄CH₃ and a (CH₂)₁₃CH₃, corresponding to the X1 and X2 groups of claimed Formula 1 structure, thereby indicating at least 13, 14, 24 carbons; paragraph [0184]).

As per claim 24, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein X1 and X2 are each independently C4-30 alkyl (the glycolipid comprises the R group (CH₂)₂₄CH₃ and a (CH₂)₁₃CH₃, corresponding to the X1 and X2 groups of claimed Formula 1 structure, thereby indicating independently C4-30 alkyl groups; paragraph [0184]).

As per claims 28/1-2, 28/4, 28/6, 28/23-24, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claims 1-2, 4, 6, 23-24, and Academia'452 further discloses wherein the compound is structure 1 (the glycolipid comprises the first structure of C1, which is identical to claimed structure 1; paragraph [0184]).

As per claim 29, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein the component is not glycolipid C34 or alpha-galactosylceramide (the glycolipid adjuvant can be 7DW8-5, C17, C23, C-30, Aluminum salt, Squalene, MF59, and QS-21; paragraph [0180]).

As per claim 30, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses 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 (the immunogenic composition further includes a pharmaceutically acceptable excipient, such as solvents, dispersion media, isotonic agents; paragraphs [0185], [0186], [0192]).

Continued Within the Next Supplemental Box

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient,
Continuation of:

Continued from previous Supplemental Box.

As per claim 31, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses further comprising an adjuvant, wherein the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, α -galactosylceramide, Aluminum salt, Squalene, MF59, or QS-21. Other examples of adjuvants in some vaccines that can be used in the composition of the present disclosure are aluminum hydroxide, aluminum phosphate, alum or 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 (suitable adjuvants include C34, 7DW8-5, C17, C23, C-30, α -galactosylceramide, Gluco-C34, Aluminum salt, Squalene, MF59, and QS-21; paragraph [0180]).

Claims 18-19, 28/18-19 lack an inventive step under PCT Article 33(3) as being obvious over Academia'452 and Academia'835, in view of the publication entitled "Glycan-Modified Virus-Like Particles Evoke T Helper Type 1-Like Immune Responses" by Alam, et al. (hereinafter "Alam").

As per claim 18, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the glycosyl group comprises mannoside. Alam discloses wherein the glycosyl group comprises a mannoside (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of wherein the glycosyl group comprises a mannoside, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising a mannosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, wherein mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claim 19, Academia'452 and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the glycosyl group comprises a mono-mannoside. Alam discloses wherein the glycosyl group comprises a mono-mannoside (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of wherein the glycosyl group comprises a mono-mannoside, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising a mono-mannosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, wherein mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claims 28/18-19, Academia'452, Academia'835, and Alam, in combination disclose the pharmaceutical formulation of claims 18-19, and Academia'452 further discloses wherein the compound is structure 1 (the glycolipid comprises the first structure of C1, which is identical to claimed structure 1; paragraph [0184]).

Claims 1-2, 4, 6, 18-19, 23-24, 28-31 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

2nd invention

Claims 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 lack an inventive step under PCT Article 33(3) as being obvious over Academia'452 in view of Alam and Academia'835.

As per claim 1, Academia'452 discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure similar to compound 12 specified in the instant PCT application, wherein the lipid nanoparticle encapsulates a payload (an immunogenic pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutically acceptable excipient, wherein the lipid is an α -GalCer analog, which would intrinsically encompass a C11 chain, and comprises the structure of C34, which has the two phenyl rings and fluorine group on one of the fatty acyl chains, as shown in the structure of compound 12 of the instant PCT application; paragraphs [0180], [0184], C1-first structure, [0185], [0202]). Academia'452 does not disclose the aryl mannoside group as shown in the structure of compound 12, and a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile. Alam discloses an aryl mannoside group as shown in the compound 12 structure (nanoparticles were generated that display an aryl mannoside group, which is identical to the structure comprised in compound 12 of the instant PCT application; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the formulation, as previously disclosed by Academia'452, for the integration of an aryl mannoside group as shown in the compound 12 structure, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising an aryl-mannosylated LNP comprising a well-defined lipid chain component, which encapsulates a therapeutic payload, such as an antigenic mRNA, wherein aryl-mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses. Additionally, Academia'835 discloses a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the

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corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 2, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue. Academia'835 discloses wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue (a modified nucleic acid molecule encoding a modified spike protein comprising one or more amino acid substitutions of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue; claims 1, 5). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid substitution of asparagine or N to glutamine or Q at N-linked glycosylation sequons or N-X-S/T, wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 4, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites. Academia'835 discloses wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites (the spike protein comprises one or more amino acid substitutions of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites; claim 1, 3, 5). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid substitution of S/T to alanine or A at O-linked glycosylation sites to eliminate O-linked glycosylation sites, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 6, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites; or the payload encoding the spike protein is an RNA comprising a nucleotide sequence set forth in SEQ ID NO: 3. Academia'835 discloses wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites (wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 4 that has 100% identity with SEQ ID NO: 4 of the instant PCT application, wherein the modified spike protein comprises a RBD lacking glycosylation sites; claim 1, 8). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a payload encoding a SARS-CoV spike protein wherein the spike protein comprises an amino acid sequence set forth in SEQ ID NO: 4, and wherein the spike protein comprises a receptor binding domain or RBD lacking at least one glycosylation sites, based on the teachings of Academia'835, as this modification would provide an immunogenic composition comprising a LNP encapsulating a payload encoding a modified SARS-CoV spike protein having a well-defined sequence and a reduced and/or deleted glycan profile, which exposes the glycan shield-masked epitopes of the spike protein, thereby increasing the antigenic presentation and the corresponding immune responses, and wherein the lipid used in the LNP, such as alpha-GalCer, further acts as an adjuvant to elicit immune responses to the immunogenic composition, thereby providing an improved vaccine against SARS-CoV infection.

As per claim 15, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein R1 comprises a formula of R2-RA-, wherein R2 is the substituted or non-substituted glycosyl group, and RA is an attachment group, and wherein the attachment group comprises an aryl. Alam discloses a formula of R2-RA-, wherein R2 is the substituted or non-substituted glycosyl group, and RA is an aryl attachment group (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of a glycosyl group comprising the formula R2-RA-, wherein R2 is the substituted or non-substituted glycosyl group, and RA is an aryl attachment group, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising an aryl-glycosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, which enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claim 18, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the glycosyl group comprises mannose. Alam discloses wherein the glycosyl group comprises a mannose (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for

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the integration of wherein the glycosyl group comprises a mannoside, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising a mannosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, wherein mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claim 19, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein the glycosyl group comprises a mono-mannoside. Alam discloses wherein the glycosyl group comprises a mono-mannoside (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of wherein the glycosyl group comprises a mono-mannoside, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising a mono-mannosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, wherein mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claim 21, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, but Academia'452 does not disclose wherein R1 is the first shown/claimed structure. Alam discloses wherein the glycosyl group comprises an aryl-mono-mannoside group which is identical to the first claimed R1 structure (nanoparticles were generated that display an aryl mono-mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the composition, as previously disclosed by Academia'452, for the integration of wherein the glycosyl group comprises an aryl-mono-mannoside group which is identical to the first claimed R1 structure, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising a aryl-mono-mannosylated LNP encapsulating a therapeutic payload, such as an antigenic mRNA, which enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

As per claim 23, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein at least one of X1 and X2 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 (the glycolipid comprises (CH₂)₁₃CH₃ corresponding to the X2 group, thereby indicating a saturated hydrocarbon chain of at least 14 carbons; paragraph [0184]).

As per claim 24, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein X1 and X2 are each independently -(CH₂)_nX₄, n is 11, and X₄ is aryloxy, or a substituted version thereof, and C14 alkyl, respectively, as shown in compound 12 (an immunogenic pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutical acceptable excipient, wherein the lipid is an alpha-GalCer analog, which would intrinsically encompass two lipid chains comprising a C11 and C14 alkyl chain, and comprises the structure of C34, which has the two phenyl rings and fluorine group on one of the fatty acyl chains, which is an aryloxy group version, as shown in the structure of compound 12 of the instant PCT application; paragraphs [0180], [0184], C1-first structure, [0185], [0202]).

As per claim 25, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein X₄ is an aryloxy, and wherein X₄ comprises to 6 substituents, selected from the group consisting of C1-6 alkyl, halogen, C1-6 alkyl halogen, and C1-6 alkoxy (an immunogenic pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutical acceptable excipient, wherein the lipid is an alpha-GalCer analog, which would intrinsically encompass two lipid chains comprising a C11 and C14 alkyl chain, and comprises the structure of C34, which has the two phenyl rings and fluorine group on one of the fatty acyl chains, which is an aryloxy group version, as shown in the structure of compound 12 of the instant PCT application; paragraphs [0180], [0184], C1-first structure, [0185], [0202]).

As per claim 26, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 25, and Academia'452 further discloses wherein X₄ is -R3-O-R4, wherein R3 and R4 are each independently aryl, each comprising to 6 substituents selected from the group consisting of C1-6 alkyl, halogen, C1-6 alkyl halogen, and C1-6 alkoxy (an immunogenic pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutical acceptable excipient, wherein the lipid is an alpha-GalCer analog, which would intrinsically encompass two lipid chains comprising a C11 and C14 alkyl chain, and comprises the structure of C34, which has the two phenyl rings (-R3-O-R4, wherein R3 and R4 are each independently aryl) and fluorine group on one of the fatty acyl chains, which is an aryloxy group version, as shown in the structure of compound 12 of the instant PCT application; paragraphs [0180], [0184], C1-first structure, [0185], [0202]).

As per claims 28/1-2, 28/4, 28/6, 28/15, 28/18-19, 28/21, 28/23-26, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claims 1-2, 4, 6, 15, 18-19, 21, 23-26, and Academia'452 further discloses wherein the compound comprises a structure similar to that of claim structure 6 or compound 12 as shown in the application specification (an immunogenic composition comprising alpha-GalCer analogs, which would intrinsically encompass two lipid chains comprising a C11 and C14 alkyl chain, and comprises the structure of C34, which has the two phenyl rings and fluorine group on one of the fatty acyl chains, as shown in claim structure 6; paragraphs [0182], [0184]). Academia'452 does not disclose the aryl mannoside group as shown in claim structure 6 or compound 12 as shown in the application specification. Alam discloses an aryl mannoside group as shown in claim structure 6 or compound 12 as shown in the application specification (nanoparticles were generated that display an aryl mannoside group; abstract; fig. 1b). It would have been obvious to a person of ordinary skill in the art, before the relevant date, to have modified the formulation, as previously disclosed by Academia'452, for the integration of an aryl mannoside group as shown in structure 6, based on the teachings of Alam, as this modification would provide an immunogenic composition comprising an aryl-mannosylated LNP comprising a well-defined lipid chain component, which encapsulates a therapeutic payload, such as an antigenic mRNA, wherein aryl-mannosylation of the LNP enhances uptake by antigen presenting cells, and improves the potency of the vaccines to elicit immune responses.

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As per claim 29, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses wherein the component is not glycolipid C34 or alpha-galactosylceramide (the glycolipid adjuvant can be 7DW8-5, C17, C23, C-30, Aluminum salt, Squalene, MF59, and QS-21; paragraph [0180]).

As per claim 30, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses 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 (the immunogenic composition further includes a pharmaceutically acceptable excipient, such as solvents, dispersion media, isotonic agents; paragraphs [0185], [0186], [0192]).

As per claim 31, Academia'452, Alam, and Academia'835, in combination disclose the pharmaceutical formulation of claim 1, and Academia'452 further discloses further comprising an adjuvant, wherein the adjuvant comprises C34, Gluco-C34, 7DW8-5, C17, C23, C30, alpha-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 or 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 (suitable adjuvants include C34, 7DW8-5, C17, C23, C-30, alpha-galactosylceramide, Gluco-C34, Aluminum salt, Squalene, MF59, and QS-21; paragraph [0180]).

Claims 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

3rd invention

Claims 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 meet the criteria set out in PCT Article 33(2)-(3).

Claim 1 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application, wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'452 discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a glycolipid compound, wherein the lipid nanoparticle encapsulates a payload (pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutically acceptable excipient, wherein the lipid is an alpha-GalCer analog; paragraphs [0180], [0184], [0185], [0202]). Academia'452 does not disclose wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application, and wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'835 discloses a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35). Academia'835 does not disclose a lipid nanoparticle formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application.

Alam discloses a nanoparticle comprising an aryl mannoside group (nanoparticles were generated that display an aryl mannoside group; abstract; fig. 1b). Alam does not specifically disclose pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application, wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'452, Academia'835, and Alam, either alone or in combination fail to disclose a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application. While Academia'452 and Alam disclose glycolipid compounds and mannoside groups, respectively, they do not specifically disclose a compound comprising the structure of compound 21. Thus, it would not have been obvious to a person of ordinary skill in the art, at the time of invention, to have implemented the pharmaceutical formulation of claim 1 because the references of record fail to disclose all the elements.

Claims 2, 4, 6, 15, 21, 23-26, 28-31 meet the criteria set out in PCT Article 33(2)-(3), because of their dependence upon positive claim 1.

Claims 1-2, 4, 6, 15, 18-19, 21, 23-26, 28-31 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

4th invention

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Claims 1-2, 4, 6, 15, 18-19, 21, 23-31 meet the criteria set out in PCT Article 33(2)-(3).

Claim 1 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of formula 1, wherein R1 is an aryl bi-mannoside group, X1 is $-(CH_2)_nX_4$, n is 11, X4 is two phenyl rings joined by an O and comprising a Fluorine group, X2 is a C13 alkyl and X3 is hydroxyl, wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'452 discloses a pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a glycolipid compound, wherein the lipid nanoparticle encapsulates a payload (pharmaceutical composition comprising an active ingredient encapsulated in a lipid nanoparticle and a pharmaceutical acceptable excipient, wherein the lipid is an alpha-GalCer analog; paragraphs [0180], [0184], [0185], [0202]); Academia'452 does not disclose wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of formula 1, wherein R1 is an aryl bi-mannoside group, X1 is $-(CH_2)_nX_4$, n is 11, X4 is two phenyl rings joined by an O and comprising a Fluorine group, X2 is a C13 alkyl and X3 is hydroxyl, wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'835 discloses a payload encoding a SARS-CoV spike protein wherein the spike protein has a reduced and/or deleted glycan profile (a pharmaceutical formulation comprising a modified nucleic acid molecule encoding a modified SARS-CoV spike protein to eliminate N-linked glycan sequons (reduced and/or deleted glycan profile), encapsulated in a LNP comprising guanidine-based polymers (plurality of lipid components), such as P1/P3, which is a bi-functional polymer, and a pharmaceutically-acceptable excipient; paragraphs [00104], [00105], [00122], [00138], [00217]; claims 1-2, 5, 30-32, 35). Academia'835 does not disclose a lipid nanoparticle formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of compound 21 specified in the instant PCT application.

Alam discloses a nanoparticle comprising an aryl mannoside group (nanoparticles were generated that display an aryl mannoside group; abstract; fig. 1b). Alam does not specifically disclose pharmaceutical formulation, comprising a lipid nanoparticle and a pharmaceutically-acceptable excipient; wherein the lipid nanoparticle is formed with a plurality of lipid components; wherein the plurality of lipid components comprises a bi-functional compound comprising the structure of formula 1, wherein R1 is an aryl bi-mannoside group, X1 is $-(CH_2)_nX_4$, n is 11, X4 is two phenyl rings joined by an O and comprising a Fluorine group, X2 is a C13 alkyl and X3 is hydroxyl, wherein the lipid nanoparticle encapsulates a payload encoding a spike protein of SARS-CoV wherein the spike protein has a reduced and/or deleted glycan profile.

Academia'452, Academia'835, and Alam, either alone or in combination fail to disclose a bi-functional compound comprising the structure of formula 1, wherein R1 is an aryl bi-mannoside group, X1 is $-(CH_2)_nX_4$, n is 11, X4 is two phenyl rings joined by an O and comprising a Fluorine group, X2 is a C13 alkyl and X3 is hydroxyl. While Academia'452 and Alam disclose glycolipid compounds and mannoside groups, respectively, they do not specifically disclose a compound comprising the structure of formula 1, wherein R1 is an aryl bi-mannoside group, X1 is $-(CH_2)_nX_4$, n is 11, X4 is two phenyl rings joined by an O and comprising a Fluorine group, X2 is a C13 alkyl and X3 is hydroxyl. Thus, it would not have been obvious to a person of ordinary skill in the art, at the time of invention, to have implemented the pharmaceutical formulation of claim 1 because the references of record fail to disclose all the elements.

Claims 2, 4, 6, 15, 21, 23-31 meet the criteria set out in PCT Article 33(2)-(3), because of their dependence upon positive claim 1.

Claims 1-2, 4, 6, 15, 18-19, 21, 23-31 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

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- External customers may access SCORE content via PAIR using the Supplemental Content tab.

●

Form Revision Date: March 1, 2019

1. Delta: SARS-CoV-2 Delta Strain Spike protein sequence from Genebank (ref: <https://viralzone.expasy.org/9556>; <https://www.ncbi.nlm.nih.gov/protein/QUD52764>);

>Delta

```
MFVFLVLLPLVSSQCVNLRTTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNG
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NCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
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2. WH: SARS-CoV-2 Wuhan-Hu-1 strain Spike protein sequence from Genebank (ref: <https://viralzone.expasy.org/resources/Coronav/Wuhan-Hu-1%5FSpike.fasta>)

>sp|P0DTC2|SPIKE_SARS2 Spike glycoprotein OS=Severe acute respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1 SV=1

>WH

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3. sequence of RBM-001 drug substance, designed/provided by Ming Wei Chen

>RBM-001

VNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDG
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CTFEYVSQPFLLMDLEGKQGNFKNLREFVFNIDGYFKIYKHTPINLVRDLPPQGSFALEPLVDLPIGINITRFQTL
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QSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGSFCTQLNRA
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4. Smg: sequence of the Smg protein described in the Huang et al 2022 Sci Transl Med paper,
provided by Dr. Che Alex Ma

>Smg

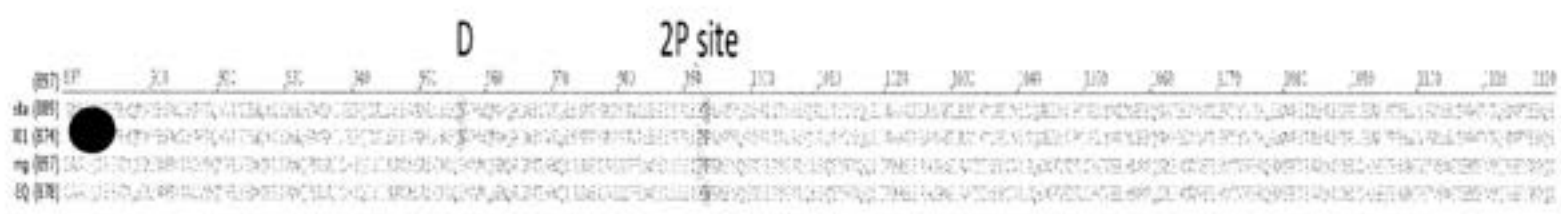
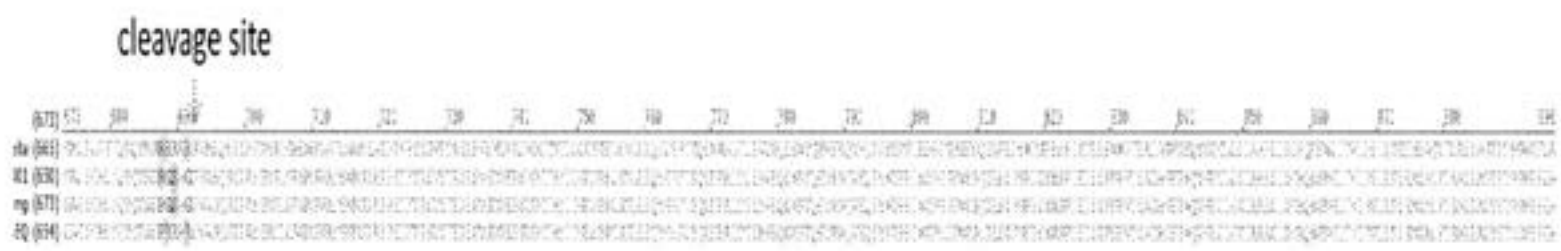
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5. SEQ: SEQ ID#1 listed in the Smg patent (A1000-00900NP_SeqListing_20230113)

>SEQ ID 1 Smg patent

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<https://www.ncbi.nlm.nih.gov/protein/QU052764>

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION # 63/549,343	RECEIPT DATE / TIME 02/02/2024 06:23:26 PM Z ET	ATTORNEY DOCKET # A1000-01400P2
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Title of Invention

LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS

Application Information

APPLICATION TYPE	Utility - Provisional Application under 35 USC 111(b)	PATENT #	-
CONFIRMATION #	7068	FILED BY	Shannon Warner
PATENT CENTER #	64196446	FILING DATE	-
CUSTOMER #	169053	FIRST NAMED INVENTOR	Chi-Huey Wong
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kay Yang

Documents

TOTAL DOCUMENTS: 5

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01400P2_ADS_20240201.pdf	8	Application Data Sheet	2224 KB
A1000-01400P2_Specification_20231009_PNAS_paper.pdf	27	-	689 KB
A1000-01400P2_Specification_20231009_PNAS_paper-SPEC.pdf	(1-22) 22	Specification	281 KB
A1000-01400P2_Specification_20231009_PNAS_paper-SPEC.pdf	(23-27) 5	Drawings-other than black and white line drawings	538 KB

31009_PNAS_paper-
DRW.NONBW.pdf

RBM-001 protein sequence analysis 01082024.pdf	3	Specification	93 KB
RBM-001 protein sequence analysis 01082024_from PPT.pdf	2	Drawings-other than black and white line drawings	227 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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331

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
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Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chi-Huey		Wong		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Rancho Santa Fe	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1		P.O. Box 8154			
Address 2					
City	Rancho Santa Fe	State/Province	CA		
Postal Code	92067	Country	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jeng Shin		LEE		
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service					
City	Taipei City	Country of Residence	TW		
Mailing Address of Inventor:					
Address 1		c/o Rock BioMedical Inc., Rm 301, Bldg. A, No. 99			
Address 2		Ln. 130, Sec. 1, Academia Rd., Nangang Dist.			
City	Taipei City	State/Province			
Postal Code	115202	Country	TW		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					
Add					

Correspondence Information:

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332

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Customer Number	169053		
Email Address	ipdocketing@prosytagroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		
Attorney Docket Number	A1000-01400P2	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	7	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☒ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
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339
333

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		
Customer Number	169053		

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☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400P2
		Application Number	
Title of Invention	LOW-SUGAR SARS-COV-2 SPIKE MRNA VACCINES WITH DELETION OF GLYCAN SHIELDS IN THE S2 OR STEM DOMAIN ELICIT BROADLY PROTECTIVE IMMUNE RESPONSES AGAINST CORONAVIRUS VARIANTS		

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Figures

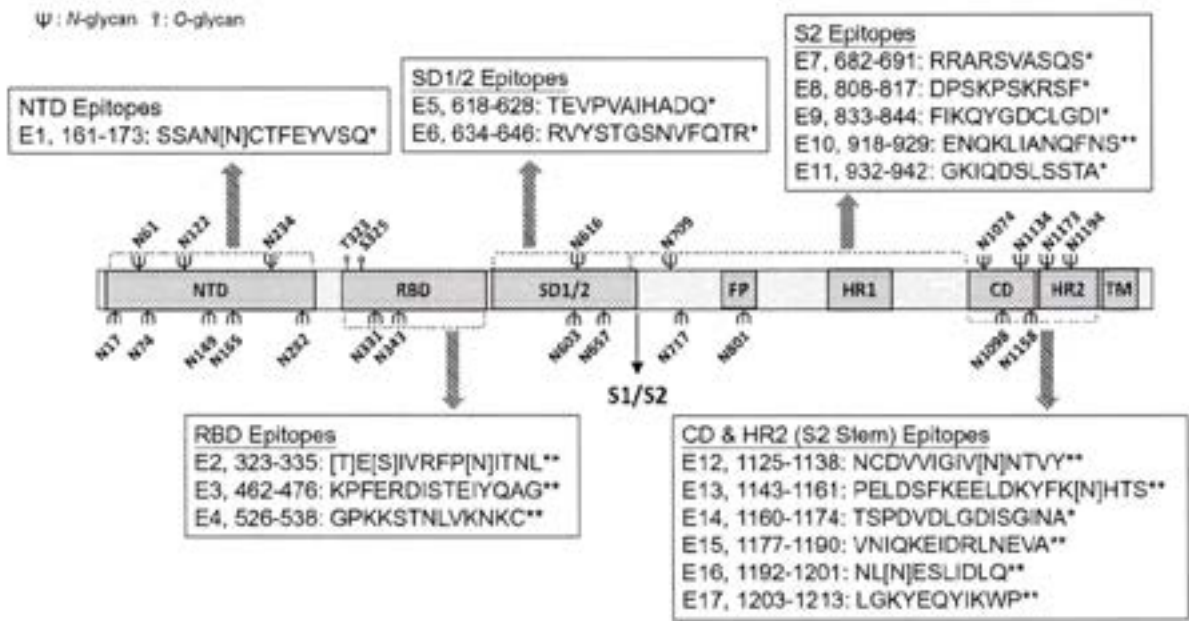


Figure 1. We have identified seventeen linear, conserved surface epitopes (E1-E17) on the SARS-CoV-2 spike glycoprotein with less than 1% residue mutation rates. All epitopes except E17 are shielded by glycans. The 11 conserved epitopes located in the S2 region have residue mutation rates of either less than 0.5% (indicated by a single asterisk) or less than 0.1% (indicated by a double asterisk, most concentrated in the stem region of the S2 domain). Removal of glycans from the S2 domain or the stem region can expose these epitopes and is useful for universal vaccine design. Note that residues in square brackets represent either N- or O-glycosites.

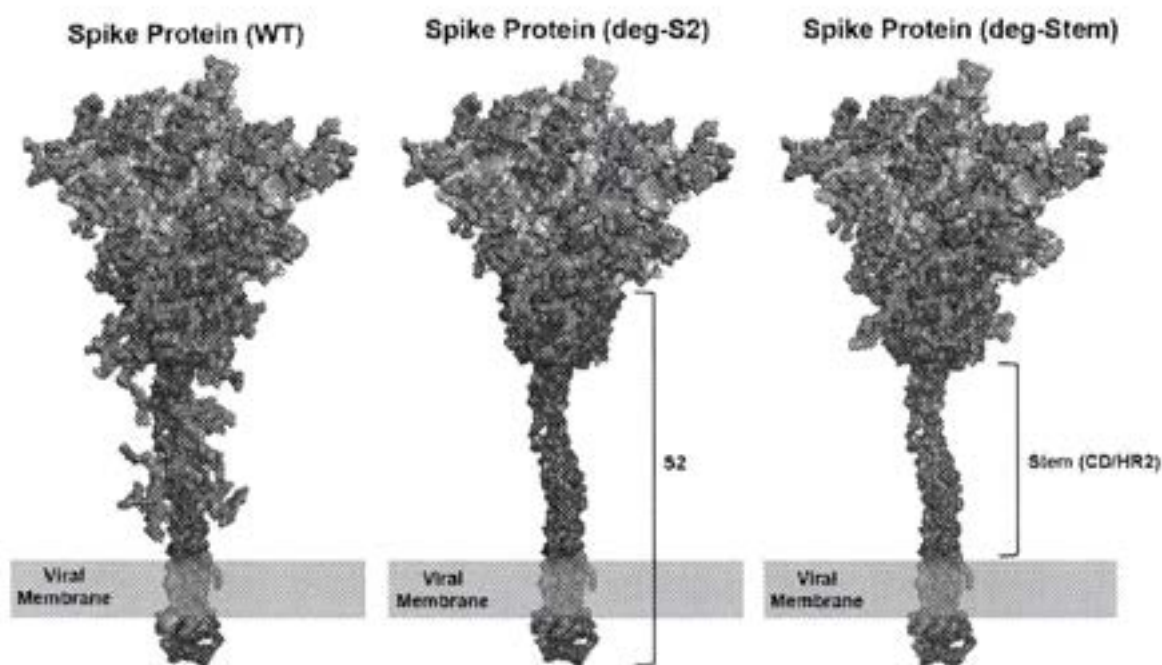


Figure 2. The 3D wild type (WT), deg-S2 (deletion of 9 glycosites), and deg-Stem (deletion of 6 glycosites) spike glycoprotein structures of SARS-CoV-2 are labeled with conserved surface regions and glycans. Gray: S1 conserved region. Dark gray: S2 conserved region. Blue: Glycans. Red: Regions are not suitable for broadly vaccine development, including mutated residues in dominant strains, residues with mutation rates of more than 1%, and buried residues. Orange: transmembrane domain.

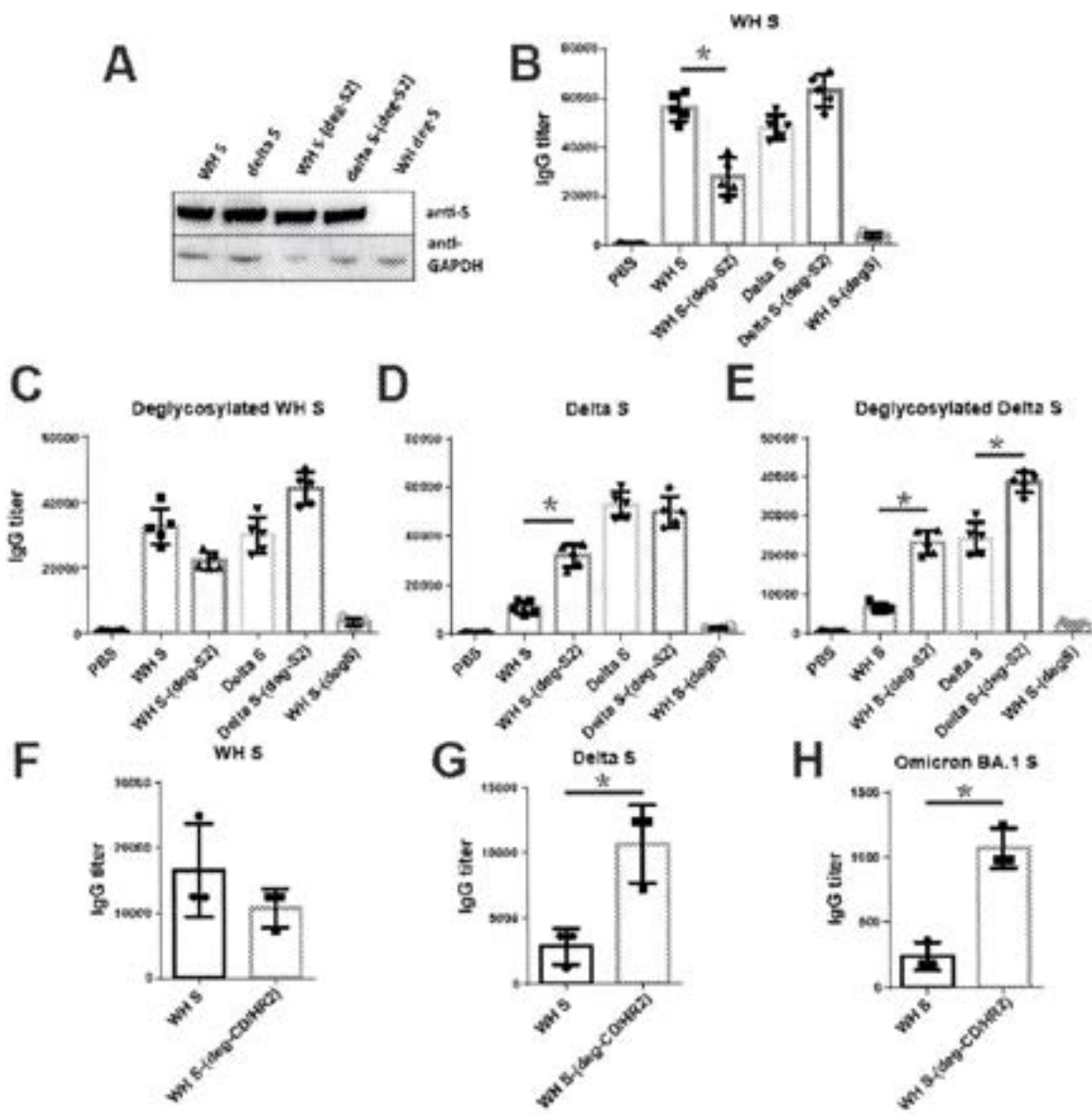


Figure 3. Effect of spike glycosylation on antibody production and specificity. (A), Analysis of S protein expression after transfection with mRNA at 48 h by Western blot. The filter was probed with anti-S and anti-GAPDH monoclonal antibodies. Humoral immune response in BALB/c mice was shown as a protein-specific IgG titer from serum against (B) WH S, (C) deglycosylated WH S, (D) Delta S, and deglycosylated Delta S

analyzed by ELISA. The protein-specific IgG titer from the mice that immunized with deglycosylated CD/HR2 S mRNA vaccine. The IgG titer against (F) WH S, (G) Delta S and (H) Omicron BA.1 S were analyzed by ELISA. B–E shows mean $\pm$ SD for five independent experiments. F–H shows mean $\pm$ SD for three independent experiments. *P < 0.001.

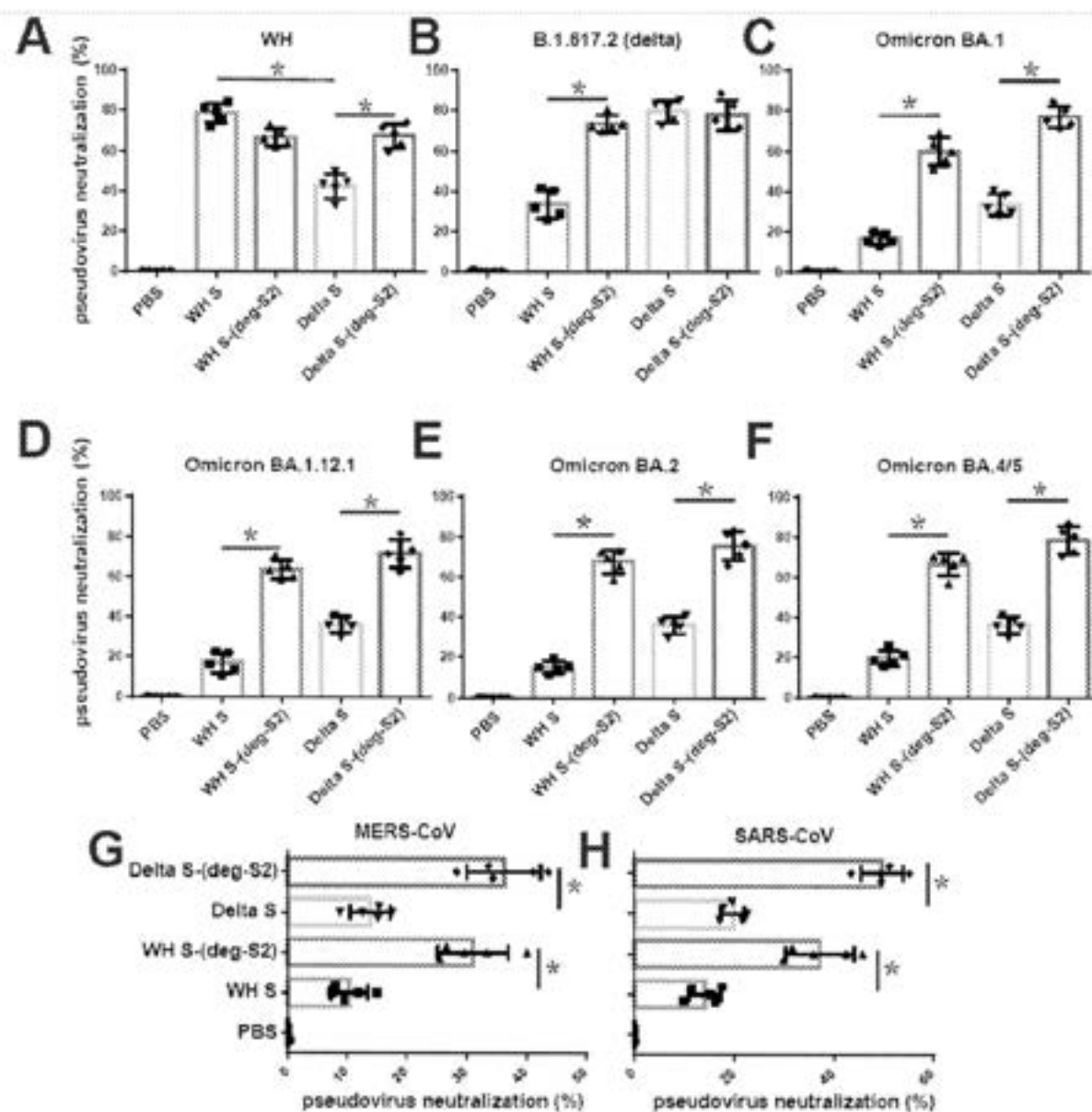


Figure 4. The breadth of mRNA vaccine protection against pseudovirus variants of coronavirus. Neutralization assay of pseudovirus variants are shown with (A) WH, (B) Delta, (C) Omicron BA.1, (D) Omicron BA.1.12.1, (E) Omicron BA.2, (F) Omicron BA.4/5. (G) MERS-CoV and (H) SARS-CoV. (A–H) show mean $\pm$ SD for five independent experiments. * $P < 0.001$.

PNAS

Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants

Chi-Huey Wong

Jeng Shin Lee

Abstract

Since the outbreak of SARS-CoV-2 in 2019, more than 15 million spike protein sequences have been identified, raising a new challenge for development of a broadly protective vaccine against the various emerging variants. We found that the virus, like most other human viruses, depends on host-made glycans to shield the conserved epitopes on spike protein from immune response and demonstrated that deletion of the glycan shields exposed highly conserved epitopes and elicited broadly protective immune responses. In this study, we identified 17 conserved epitopes from 14 million spike protein sequences and 11 of the conserved epitopes are in the S2 domain, including the 6 most conserved epitopes in the stem region. We also demonstrated that deletion of the glycosites in the spike mRNA S2 domain or the stem region exposed the highly conserved epitopes and elicited broadly protective immune responses,

particularly CD-8⁺ T cell response against various SARS-CoV-2 variants, and other human coronaviruses including MERS and SARS viruses.

Significance Statement

In an effort toward development of a universal vaccine targeting the highly conserved epitopes on SARS-CoV-2 and variants, we examined 14 million sequences of spike proteins reported by Global Initiative on Sharing All Influenza Data (GISAID) and identified 17 linearly conserved epitopes, with 11 located in the S2 domain including the 6 most conserved epitopes in the stem region. Deletion of the glycosites in the S2 domain or the stem region exposed the glycan-shielded conserved epitopes and elicited broadly protective immune responses against SARS-CoV-2 variants and other human coronaviruses, including MERS and SARS viruses.

Introduction

Since the outbreak of Severe Acute Respiratory Syndrome Virus-2 (SARS-CoV-2) in 2019 in Wuhan, China, the virus has spread rapidly around the world and continued to change with mutation. As of February 15, 2023, the virus and its variants infected more than 650 million people and caused more than 6.6 million deaths (1, 2). A great deal of global efforts has been directed toward development of effective means to contain the pandemic, and among them, vaccination has been the most effective. Of the many types of vaccine candidates available, the successful development of mRNA vaccines represents a breakthrough in the field (3). After administration of an mRNA vaccine, it is translated *in vivo* to the real protein antigen to elicit immune responses, so

there is no need to manufacture the protein vaccine *in vitro*. However, mRNA vaccines are unstable and must be modified and formulated with lipid nanoparticles (LNPs) for storage and administration (3). Currently, all mRNA vaccines are developed based on the surface spike protein of the virus as an immunogen. However, various emerging variants, such as Omicron subvariants, are able to significantly escape the immune responses to the spike protein (4). Therefore, to develop a broadly protective vaccine against the current and upcoming variants, it is necessary to analyze the huge number of SARS-CoV-2 S protein sequences in the GISAID (Global Initiative on Sharing All Influenza Data) database (5, 6) and identify the conserved sequences as targets for development of vaccines with broad protection and long-acting immunity.

Our previous study showed that human viruses are all coated with host-made sugars to facilitate infection and to shield the conserved epitopes from immune response, and deletion of the glycan shields from spike protein exposed highly conserved epitopes and elicited broadly protective immune responses (7). In addition, the SARS-CoV-2 mRNA vaccine with deletion of glycosites in the S2 domain of the spike protein elicited broadly protective antibody and memory CD8⁺ T-cell responses against the highly conserved epitopes shielded by glycans (8, 9). This low-sugar vaccine approach, conceived in 2009 for universal influenza vaccine development (10), was also demonstrated as an effective strategy for development of a broadly protective HIV vaccine (11), and the approach is also consistent with the recent report that the spine helix in S2 (the stem) is highly conserved and can be a possible epitope for universal vaccine design (12). To continue this line of study, we identified 17 conserved

epitopes from more than 14 million S protein sequences reported to GISAID, including those with less than 1% residue mutation rates. Of these 17 conserved epitopes, 11 are in the S2 domain, including 6 in the CD/HR2 (stem) region. In this and previous analyses of the spike protein sequences, we found that the 6 linear epitopes in the stem are the most conserved but shielded by the glycans from the 6 *N*-glycosites in this region. The other conserved epitopes are spread in different domains. Based on this finding, we believe that deletion of the glycans in the stem or the S2 domain will expose the highly conserved epitopes and elicit broadly protective immune responses. In this study, we showed that the spike mRNA vaccine from the Wuhan strain or the Delta strain with deletion of the 9 glycosites in the S2 or the 6 glycosites in the stem region elicited more broadly protective immune responses as compared to the unmodified mRNA vaccine. To further understand the origin of the broad protection, we found that glycosylation affects protein stability and cellular immune response, and in addition to the variants and subvariants of SARS-CoV-2, the glycosite-deleted mRNA vaccine is capable of eliciting cross-reactive immune responses against not only SARS-CoV-2 variants but also alpha and beta coronaviruses including MERS and SARS viruses.

Results

Seventeen linearly conserved epitopes are identified in the S protein of SARS-CoV-2. Previously, we identified 12 conserved epitopes from 6 million S protein sequences, including those with residue mutation rates less than 0.1%; 7 of the 12 conserved epitopes are in the receptor binding domain (RBD) and 5 in the S2 domain (8). However, 4 of the 7 conserved epitopes in the RBD have mutations in Omicron

subvariants, thus reducing the effectiveness of vaccines and therapeutic antibodies that target the RBD domain (13). In this study, we used a less than 1% mutation rate to identify 17 conserved epitopes from 14 million S protein sequences.

Simulated 3D structures of S proteins, with and without the 66 N-glycans and 6 O-glycans were obtained based on our previous research (7). By calculating the relative solvent accessibility (RSA) of each amino acid of the spike protein and comparing the two types of structures, three states of each residue designated as exposed, shielded by glycans, and buried, can be identified. Mutation information provided by the GISAID database was then used to identify which amino acids are conserved and not affected by mutations in dominant strains, including the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, BA.2.12.1, BA.2.75.*), and top-ranked emerging variants (e.g., XBB.1.5, XBB.1.16, XBB.1.9.1, BQ.1.1, EG.5 etc.) reported by GISAID. The above information was integrated to confirm that the sequences identified are conserved with continuous amino acid sequences (10-20 in length). The IEDB (The Immune Epitope Database) (14) was also used to confirm that these sequences were epitopes (please check Materials and Methods for more details).

Fig. 1 shows the seventeen linearly conserved epitopes identified in this study. One of them (E1) is in the NTD, three (E2-E4) in RBD, two (E5-E6) in SD1/2, and eleven (E7-E17) in the S2 domain, including 6 in the CD/HR2 (the stem) region, which are the most conserved epitopes in this analysis. Except for the E17, all conserved epitopes were shielded by glycans, suggesting that during the antigen presentation process, the glycans may not be completely processed and thus may shield the conserved epitopes

from immune cell recognition. In the figure, single asterisks indicate the eight conserved epitopes with less than 0.5% mutation rate of each amino acid in the epitopes and double asterisks indicate the nine highly conserved epitopes with less than 0.1% mutation rate of each residue in the epitopes. Interestingly, 6 of these highly conserved epitopes are concentrated in the stem (E12-E17) and 3 in the RBD (E2-E4). It is also noted that E3 is in the receptor-binding motif (RBM, 438-506) of RBD, the site for S protein binding to ACE2. The conservation of E3 suggests that it is probably essential for pathogen-host interaction.

We then divided all residues of S protein into three categories for analysis: buried, shielded, and exposed residues (the residue number in each category is 851:172:250). The average mutation rates of the three categories were 0.84%, 1.67%, and 3.66%, and the standard deviations were 5.90%, 8.33%, and 11.58%, respectively. Buried residues are those not easily recognized by the immune system and have the lowest mutation rate, followed by shielded residues and the highest exposed residues. Based on this analysis, the conserved S protein epitopes are concentrated in the stem region of S2 as shown in Fig. 2. Thus, deleting the 9 glycosites in S2 or the 6 glycosites in the stem to generate the low-sugar spike protein or its mRNA as vaccine is expected to better expose the conserved and glycan-shielded epitopes to the immune system and thus elicit broadly protecting immune responses against the conserved epitopes.

SARS-CoV-2 and SARS-CoV share the same epitopes in the stem. We were curious whether these conserved epitope sequences in the S protein of SARS-CoV-2 were also found in the S protein of SARS-CoV. Therefore, we performed a pairwise sequence

alignment between the S protein sequences of SARS-CoV-2 and SARS-CoV. The result (Fig. S1) showed that 5 of the 17 conserved SARS-CoV-2 epitopes are identical to SARS-CoV (E13-E17), and interestingly, they are all located in the stem region (CD and HR2). Among the 6 conserved epitopes (E12-E17) in the stem of the SARS-CoV-2 S protein, 5 epitopes (E12-E16) are shielded by glycans. Moreover, of these 6 epitopes (E12-E17), E14 has residue mutation rates of less than 0.5%, while the other four epitopes have residue mutation rates of less than 0.1%. This indicates that the conservation of the stem is relatively high, making it a suitable target for development of broadly protective vaccines. We also examined the E12 epitope located in the CD region of SARS-CoV-2 S protein and found that the sequence differed from SARS-CoV by only one residue (DVVIGI[V/I] NNTVY). Thus, we concluded that the vaccine with deletion of S2 or stem glycosites might also be effective against SARS-CoV, and this indeed was supported by the finding in our previous study of mono-GlcNAc decorated SARS-CoV-2 S protein (7).

The spike mRNA vaccine with deletion of glycosites in S2 increases the breadth of antibody neutralization. Since the spike mRNA vaccine from the Wuhan (WH) strain with deletion of the 9 glycosites in S2 provided a broad protection against SARS-CoV-2 variants (8), we used Wuhan (WH) and Delta variants (15) as models for comparative study. To study how the mRNA vaccine of S protein with deletion of glycosites in S2 affected S protein expression and immune response, we deleted the *N*-glycosites (from N to Q in the N-X-S/T sequon) where the conserved epitopes were shielded by glycans. After confirming the expression of the prefusion S protein in the

HEK293 cell line (Fig. 3A), the mRNA of WH strain S protein with deletion of all *N*- and *O*-glycosites (deg-S) did not express the S protein well. The mRNA that encoded the S protein, the S protein with deletion of S2 glycosites, or the S protein with deletion of all glycosites was then encapsulated in lipid nanoparticles (LNPs) to form mRNA-LNP for immunization in mice. Compared to the unmodified spike mRNA vaccine, sera from mice immunized by the spike mRNA with deletion of all S2 *N*-glycosites in WH S (WH S-(deg-S2)) elicited a slightly lower (50 -67%) IgG titer against the fully glycosylated WH S (Fig. 3B) or deglycosylated WH S protein (Fig. 3 C) but a higher (7 fold) IgG titer against the fully glycosylated Delta S (Fig. 3D) or deglycosylated Delta S protein in enzyme-linked immunosorbent assay (ELISA) (Fig. 3E). Similarly, the Delta spike mRNA vaccine with deletion of glycosites in S2 (Delta S-(deg-S2)) elicited a slightly lower IgG titer against the fully glycosylated Delta S protein (Fig. 3D) but a higher IgG titer against the fully glycosylated or deglycosylated WH S (Fig. 3B, C) and the deglycosylated Delta S protein (Fig. 3E). A similar result was observed in the case of spike mRNA vaccine with deletion of the 6 glycosites in the stem region (WH S-(deg-CD/HR2)) (Fig. 3F-H) (16). The mice immunized with WH S-(deg-CD/HR2) mRNA vaccine induced a stronger antibody response (10 fold) with a higher IgG titer against the S protein of Delta (Fig. 3G) and Omicron BA.1 (Fig. 3H) variants as compared to the unmodified mRNA, suggesting that deleting the glycan shield in the stem would enhance antibody response to the stem with cross-reactivity against the S protein of other variants containing the conserved epitopes.

To analyze the effect of S2 glycosite deletion on the neutralization activity of antibodies generated from immunized mice, the pseudovirus neutralization assay was performed, and the result showed that the WH and Delta mRNA vaccines with deletion of glycosites in S2 generated antibodies with slightly reduced 10 % of neutralization activity against WH or Delta pseudovirus, respectively (Fig. 4A, B), but with increased 5-8 fold of neutralization activity against the Omicron variants including BA.1 (Fig. 4C), BA.1.12.1 (Fig. 4D), BA.2 (Fig. 4E), BA.4 and BA.5 (Fig. 4F), than the unmodified mRNA. This finding was consistent with our previous study that WH mRNA vaccine with deletion of all S2 N-glycosites induced a broader protection against the alpha, beta, gamma and delta strains, perhaps due to the conserved antibody response to the conserved epitopes after deletion of the shielded glycans (8).

Glycosylation affects protein stability and cellular immune response.

To characterize the T cell response, splenocytes from immunized mice were isolated and incubated with the peptide pool of WH S, RBD, and S2 protein to measure granzyme B (GrzB)-secreting T cells by enzyme-linked immune absorbent spot (ELISpot) analysis. It was found that the mRNA vaccine with deletion of S2 glycosites induced more GrzB-secreting cells than the unmodified spike mRNA of WH and Delta (Fig. S2A-C). The Delta S-(deg-S2) with more stable S protein expression than that of the WH S-(deg-S2) showed a weaker T cell response (Fig. S3). Interestingly, the WH S-(deg-S) vaccine with all glycosites deleted showed the lowest stable protein expression but the highest T cell response. These results indicate that unlike the case *in vitro*, the stability and

integrity of the S protein generated *in vivo* affects T cell response and the misfolded S protein seemed to induce a stronger T cell response.

The S2 glycosite-deleted mRNA vaccine elicited cross-reactive immune response against alpha and beta coronaviruses, as well as MERS and SARS viruses. To investigate whether the antibodies induced by the spike mRNA vaccine with deletion of S2 glycosites provide a protection against human alpha and beta coronaviruses since the S2 domain and the stem region contain more conserved epitopes than the other domains among alpha and beta coronaviruses, SARS-CoV-2 variants and MERS as well as SARS virus (12, 17), it was shown that sera from mice immunized with the S2-glycosite deleted spike mRNA had higher (2-3 fold) IgG titers against human alpha (HCoV-NL63 (Fig. S4A) and HCoV-229E (Fig. S4B)) and beta (HCoV-HKU1 (Fig. S4C), HCoV-OC43 (Fig. S4D), MERS-CoV (Fig. S4E) and SARS-CoV (Fig. S4F)) coronaviruses, and increased 6-8 fold of neutralization activity against MERS-CoV (Fig. 4G) and SARS-CoV (Fig. 4H) in pseudovirus neutralization assay, suggesting that the spike mRNA vaccine with deletion of glycosylation on S2 provides broad protection against pan-coronaviruses.

Discussion

We have identified 17 conserved epitopes in the SARS-CoV-2 S protein, and 11 of which (more than 60%) are in the S2 region including 6 in the stem region, and 5 of the 6 conserved stem epitopes are also conserved in the stem of SARS-CoV, MERS-CoV and human alpha and beta coronaviruses. In our previous analysis of 4 million S protein sequences, seven conserved epitopes were found in the RBD; however, four of these

seven conserved epitopes, including 331-346, 368-378, 441-450, and 458-470 have mutations in the Omicron variants. In contrast, the 11 conserved epitopes in the S2 domain, especially the 6 in the stem, are still highly conserved and are more reliable as targets for vaccine development. We analyzed the mutations in the S protein O-glycosites and found that the mutation rates of T323 and S325 were relatively low, only 0.09% and 0.01%, respectively. Regarding the *N*-glycosites, we investigated if mutation of any of the 22 *N*-glycosite sequons (N-X-S/T) affected *N*-glycosylation and found that the T19R of Delta and T19I of Omicron mutations (BA.2, BA.2.12.1, BA.2.75.*, BA.4, BA.5, BQ.1.1, XBB.1.5, XBB.1.16, etc.) changed the sequons from NLT to NLR and from NLT to NLI respectively and consequently caused a loss of *N*-glycosylation at the nearby *N*-glycosite N17. Other sequons, including those in S2 and stem, were not affected significantly, so deleting the glycans from S2 or stem exposed the conserved epitopes and elicited broadly protective immune responses. In addition, recent studies have shown that the fusion inhibitors of the pan-coronavirus are also effective against SARS-CoV-2 (18), and human antibodies recognizing the fusion peptide and stem provide a broad protection against coronaviruses (12, 17, 19). These observations are consistent with our finding that the S2 domain or the stem of coronavirus is an excellent target for vaccine development.

Repeated immunization with homologous antigens, such as in the case of influenza A vaccine, may increase antibody responses to the conserved epitopes, albeit with glycan shield and provide cross-reactivity against heterologous strains (20). People receiving the third dose of the SARS-CoV-2 mRNA vaccine (BNT162b2 manufactured

by Pfizer-BioNTech and mRNA-1273 manufactured by Moderna based on the WH strain) showed protection against variants such as Omicron variants at least transiently, and cross-reactivity against heterologous strains with increased antibody titers (21). Interestingly, repeated SARS-CoV-2 WH S vaccination also elicited neutralizing antibodies targeting the stem region (12, 17, 22). However, immunization with spike mRNA vaccine with deletion of S2 glycosites elicited a stronger antibody and T cell response against pan-coronaviruses, suggesting that the induced immune responses target the conserved epitopes in the S2 region. In addition, the WH spike mRNA vaccine with deletion of the 6 glycosites in the stem also induced an antibody response with increased IgG titer against the S protein of Delta and Omicron variants, suggesting that the SARS-CoV-2 spike mRNA vaccine with deletion of stem glycosylation elicits antibodies that recognized the conserved epitopes in the stem (Fig. S2). Under the selective pressure from vaccines, glycosylation on the conserved epitopes of S protein may play an essential role in maintaining the proper tertiary and quaternary structures and simultaneously shielding the conserved epitopes from immune response. The highly conserved epitopes mainly located in the stem region of SARS-CoV-2 are also highly conserved among coronaviruses of the four genera. Therefore, neutralizing antibodies targeting the conserved epitopes in the stem should provide cross-reactive protection against pan-coronavirus (7, 8, 15, 16, 22). On the other hand, deletion of the glycan shields in the highly conserved stem domain or the S2 domain to generate low-sugar vaccines should better expose the highly conserved epitopes and elicit enhanced and broadly protective immune responses.

The serum from the mice immunized with the SARS-CoV-2 S vaccine was overall similar to human convalescent serum (23), and that from SARS-CoV-2 S2 DNA-vaccinated mice reacted strongly with an epitope in the HR2 and membrane-proximal region that was highly conserved among SARS-CoV-2 variants (7, 24). However, using the recombinant WH S2 protein without glycosylation produced by *Escherichia coli* failed to induce neutralizing antibodies against SARS-CoV-2 WH or Omicron S pseudovirus, perhaps due to the conformation change *in vitro* (24). However, the SARS-CoV-2 spike mRNA vaccines from the WH and Delta S mRNA with deletion of glycosites in S2 or stem elicited enhanced neutralizing antibody and CD8⁺ T-cell responses against SARS-CoV-2 variants and other coronaviruses, suggesting that the misfolded S protein generated *in vivo* from the mRNA with deletion of glycan shields can still be processed to elicit immune responses.

T-cell response prevented SARS-CoV-2 infection from progressing to severe conditions (25–27). In addition, CD8⁺ T cell response induced by prior infection provided approx. 80%-95% protection against SARS-CoV-2 variant reinfections for more than 8 months (9, 28, 29). In this study, we have demonstrated that the SARS-CoV-2 spike mRNA with deletion of glycosites in the S2 or stem domain reduced the stability of S protein, thereby triggering a strong memory CD8⁺ T cell induction.

Human viruses, including influenza A virus (IAV) and SARS-CoV-2 use host-made glycans to facilitate infection and to shield the conserved epitopes on the virus surface from immune response. Vaccination with mono-glycosylated hemagglutinin (HA) or inactivated IAV induced cross-strain protection against influenza virus infections (10, 30-

31). Interestingly, the HA stem-specific antibodies are broadly protective against most type A influenza viruses (32, 33), and previous studies showed that deletion of the glycans in HA elicited a high level of stem-specific antibodies (34-37) and T cell response (37). Based on the study of influenza and SARS-CoV-2, this low-sugar vaccination strategy is expected to be a general and effective approach to development of universal vaccines against other human viruses.

Materials and Methods

Conserved Epitope Identification. The 3D structural models of the SARS-CoV-2 S protein with representative glycan profiles were obtained in our previous work (7). The probe radius of 7.2Å, mimicking the hypervariable loops in the complementarity determining region of antibodies, was used in the FreeSASA program (38) to calculate each RSA of residue in S protein, both with and without representative glycans. The classification of exposed/buried residues was consistent with the definition in the previous study by Kajander *et al.* (39). Glycans are considered to provide shielding for the residues with buried states in models with glycans whereas these same residues have exposed states in models without glycans. A total of 14,624,495 SARS-CoV-2 S protein sequences and their variant information were extracted from the GISAID database (version: March 3, 2023) for amino acid mutation rate calculation. The linear conserved epitopes in this study are 10-20 contiguous amino acids in length and the state of the residues are mostly exposed or exposed but shielded by glycans. All amino acid mutation rates in the conserved epitope sequences should be <1% and not

affected by mutations in the dominant virus strains and the top-ranked emerging variants. The dominant virus strains were defined as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, BA.2.12.1, BA.2.75.*). The top-ranked emerging variants (spread) reported by GISAID are BA.2.47, BQ.1, BQ.1.1, BQ.1.1.28, BQ.1.1.32, CH.1.1.3, EG.5.1, EL.1, EU.1.1, FD.1.1, XBB.1.16, XBB.1.16.6, XBB.1.17.1, XBB.1.5, XBB.1.5.10, XBB.1.5.59, XBB.1.9.1, XBB.1.9.2, XBB.2.3, XBB.2.3.3, XBB.2.3.8, and XBF. (version: February 8, 2023, April 24, 2023, June 13, 2023, and August 19, 2023). All variants are double-confirmed by GISAID and CoV-SPECTRUM (40). All conserved sequences are confirmed by IEDB as epitopes with 100% concordance or as their subsequences.

Cell lines. The Human embryonic kidney cells (HEK293) and Huh7 human hepatoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Rockville, MD) with 10% heat-inactivated fetal bovine serum (FBS) (Thermo Scientific) and antibiotics (100 U/ml penicillin G and 100 gm/ml streptomycin).

Antibodies and proteins. The rabbit anti-SARS-CoV-2 S polyclonal antibody was purchased from ABclonal. SARS-CoV-2 full-length WH and Delta S proteins (293T cell expressed) were purchased from Royez. HCoV-NL63, HCoV-229E, HCoV-HKU1, and MERS-CoV spike protein were purchased from Sino Biologicals (Beijing, China). HCoV-OC43 spike protein was obtained from Acrobiosystems. SARS-CoV spike protein was purchased from Biotechne. Mouse monoclonal anti-GAPDH was obtained from Millipore. To obtain the deglycosylated S protein, WH or Delta S proteins were deglycosylated in a buffer solution with PNGase F (Sigma) at 37°C for 24 h in the dark. After deglycosylation, samples were purified and checked by Western blot (7, 34).

mRNA vaccine of deglycosylated S protein and formulation. The pre-fusion state of the S, the codon-optimized S gene of SARS-CoV-2 was synthesized by GenScript and cloned into pVax or pMRNATM, and was stabilized by proline substitutions at positions K968 and V969 (S-2P) (41). To delete the *N*-glycosites, the putative sequon N-X-S/T was changed to Q-X-S/T by using site-directed mutagenesis on the S-2P expression plasmid. For the in-vitro transcription, the linear DNA with the T7 promoter, 5' untranslated region, 3' untranslated region, S-2P, and poly(A) tail signal sequence was amplified by using TOOLS Ultra High Fidelity DNA Polymerase (BIOTOOLS Co., Ltd., Taipei, Taiwan) with 1 μ l of the DNA template in an mMESSAGE mMACHINE[®] Kit (Thermo Scientific) at 37°C for 1hr according to the manufacturer's protocol. The mRNA was purified by an RNA cleanup kit (BioLabs), according to the manufacturer's protocol, and stored at -80°C until further use. For the formulation mRNA-LNP, mRNA was encapsulated in LNP using a self-assembly process in which an aqueous solution of mRNA at pH 4.0 was rapidly mixed with an ethanolic lipid mixture containing ionizable cationic lipid, phosphatidylcholine, cholesterol, and polyethylene glycol-lipid (8, 42). The compositions of LNP were DSPC (Sigma), cholesterol (Sigma), DOTAP (Sigma), and DMG-PEG 2000 (Sigma). The mRNA-LNP was characterized and subsequently stored at -80°C at a concentration of 1 mg/ml. After HEK293 cells were transfected with 10 μ g of mRNA-LNP in six wells of a plate at 48 hrs, the total cell lysate was collected to monitor the expression of S by western blot (8).

Animals and immunizations. BALB/c mice aged 6-8 weeks old ($n = 5$) were immunized intramuscularly with 50 μ g mRNA-LNP in PBS with 300 mM sucrose.

Animals were immunized at week 0, boosted with a second vaccination at week 2, and

serum samples and spleens were collected from each mouse one week after the booster immunization. The animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica (8).

Serum IgG titer measure. Anti-S protein ELISA was used to determine IgG titer. Plates were coated with 50 ng/well of variant S protein as shown in Fig. 3 and S3, and then blocked with 5% skim milk. The serum from immunized mice and HRP-conjugated secondary antibodies were sequentially added. Peroxidase substrate solution (TMB) and 1M H₂SO₄ stop solution were used and absorbance (OD 450 nm) was read by a microplate reader (8).

Pseudovirus neutralization assay for serum study. SARS-CoV-2 pseudovirus variants were constructed by the RNAi Core Facility at Academia Sinica using the procedure described previously (8). The pseudotyped lentivirus was then stored at -80°C. To estimate the lentiviral titer by AlarmaBlue assay (Thermo Scientific), the transduction unit (TU) of pseudotyped lentivirus was estimated by using a cell viability assay. HEK-293T cells expressing the human ACE2 gene were plated on a 96-well plate one day before lentivirus transduction. To determine the titer of pseudotyped lentivirus, different amounts of lentivirus were added into the culture medium containing polybrene (final concentration 8 µg/ml) (Sigma), and spin infection was carried out at 1,100 xg in 96-well plate for 30 min at 37°C. After incubation for 16 h, the culture medium containing virus and polybrene was removed and replaced with fresh complete DMEM containing 2.5 µg/ml puromycin (Sigma). After treating puromycin for 48 h, the culture medium was removed, and the cell viability was detected by using AlarmaBlue reagents according to the manufacturer's instructions. The survival rate of uninfected

cells was set as 100%, and the virus titer was determined by plotting the survival cells versus the diluted viral dose (8). The SARS-CoV and MERS-CoV pseudoviruses were purchased from eEnzyme.

For neutralization assay, heat-inactivated sera or antibodies were serially diluted with desired dilution 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 or Huh7 (for MERS-CoV pseudovirus) 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 post-infection and continuously cultured for another 48 h. The expression level of the luciferase gene was determined by using 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 and the calculation formula as shown below: $(RLU_{control} - RLU_{Serum}) / RLU_{control}$ (7, 8).

Measurement of GrzB-secreting cells. A total of 5×10^5 splenocytes from immunized mice were *ex vivo* restimulated with full-length WH S, RBD, and S2 peptide mix (0.1 µg/ml final concentration per peptide) (Sino Biologicals) in the GrzB ELISpot assays (R&D Systems) according to the manufacturer's instructions and spots were counted (8).

DNA plasmid transfection and MG132 treatment. After the HEK293 cell was seeded in the 6 well plates, cells were transfected with 3 µg of each plasmid by TransIT®-LT1 Transfection Reagent (Mirus) and then incubated with 1 µM MG-132

(MedChemExpress) or DMSO at 37°C for 24 h. The total lysate was collected and the variant S expression was analyzed by western blot (8).

Statistics and reproducibility. All data were presented as means $\pm$ standard error of the mean. The numbers of samples and replicates of experiments were shown as mentioned in the figure legends. Comparisons between groups were determined using the student's t-test. Differences were considered significant at *P < 0.001, **P < 0.05. All data were analyzed using GraphPad Prism 6 software.

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Title of Invention

METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

Application Information

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CUSTOMER #	169053	FIRST NAMED INVENTOR	Chi-Huey WONG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Wade Lin

Documents

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generatedADS64981744.pdf	6	Application Data Sheet	137 KB
A1000-01100P32_20240405_Spec_TBF.pdf	74	-	1099 KB
A1000-01100P32_20240405_Spec_TBF-SPEC.pdf	(1-59) 59	Specification	866 KB
A1000-01100P32_20240405_Spec_TBF-CLM.pdf	(60-73) 14	Claims	468 KB

A1000- (74-74) 1 Abstract 95 KB
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A1000- 10 Drawings-only black and white 2009 KB
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as

described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Data Sheet 37 CFR 1.76

The Application Data Sheet is part of the provisional or non-provisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.

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Application Information

Customer number 169053 -

Correspondence address ---

Title of invention METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

Attorney docket number A1000-01100P32

Entity status Small

376
374

Application type Provisional Application under 35 USC 111(b)

Subject matter Utility

Total number of drawing sheets 10

Suggested figure for publication ---

Filing by reference No

Publication request Normal eighteen-month publication

Representative Information

of representatives: 1

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).

Customer number 169053

Domestic Benefit/National Stage Information

of benefit claims: 0

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c), 386(c), or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be part of the specification.

 Data was not provided for this section.

Foreign Priority Information

of foreign priority claims: 0

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the Application Data Sheet constitutes the claim for priority as required by

35 U.S.C. 119 (b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(j)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

 Data was not provided for this section.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

Checking this box will cause the application to be examined under the first inventor to file provisions of the AIA.

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 2016, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization or Opt-Out of Authorization to Permit Access

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant must opt-out of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE:
This section of the Application Data Sheet is ONLY reviewed and processed with the INITIAL filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

Priority Document Exchange (PDX)

Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant

application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h) (1).

Search Results from U.S. Application to EPO

Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

- B. The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

- ☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.
- ☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with the search results from the instant application.

NOTE:

Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Applicant Information

of applicants: 1

The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46.

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Applicant	Organization
Applicant type	Person to whom the inventor is obligated to assign

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Fax: ---
Email: ---

Assignee Information including Non-Applicant Assignee Information

of assignees: 0

An assignee-applicant identified in the "Applicant" section will appear on the patent application as an applicant.

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Data was not provided for this section.

Signature

NOTE:

This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c)

This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, all joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of all joint inventor-applicants.

See CFR 1.4(d) for the manner of making signatures and certifications.

Signature	First name	Last name	Registration #	Date
Wade Lin	Wade	Lin	L1383	04/05/2024

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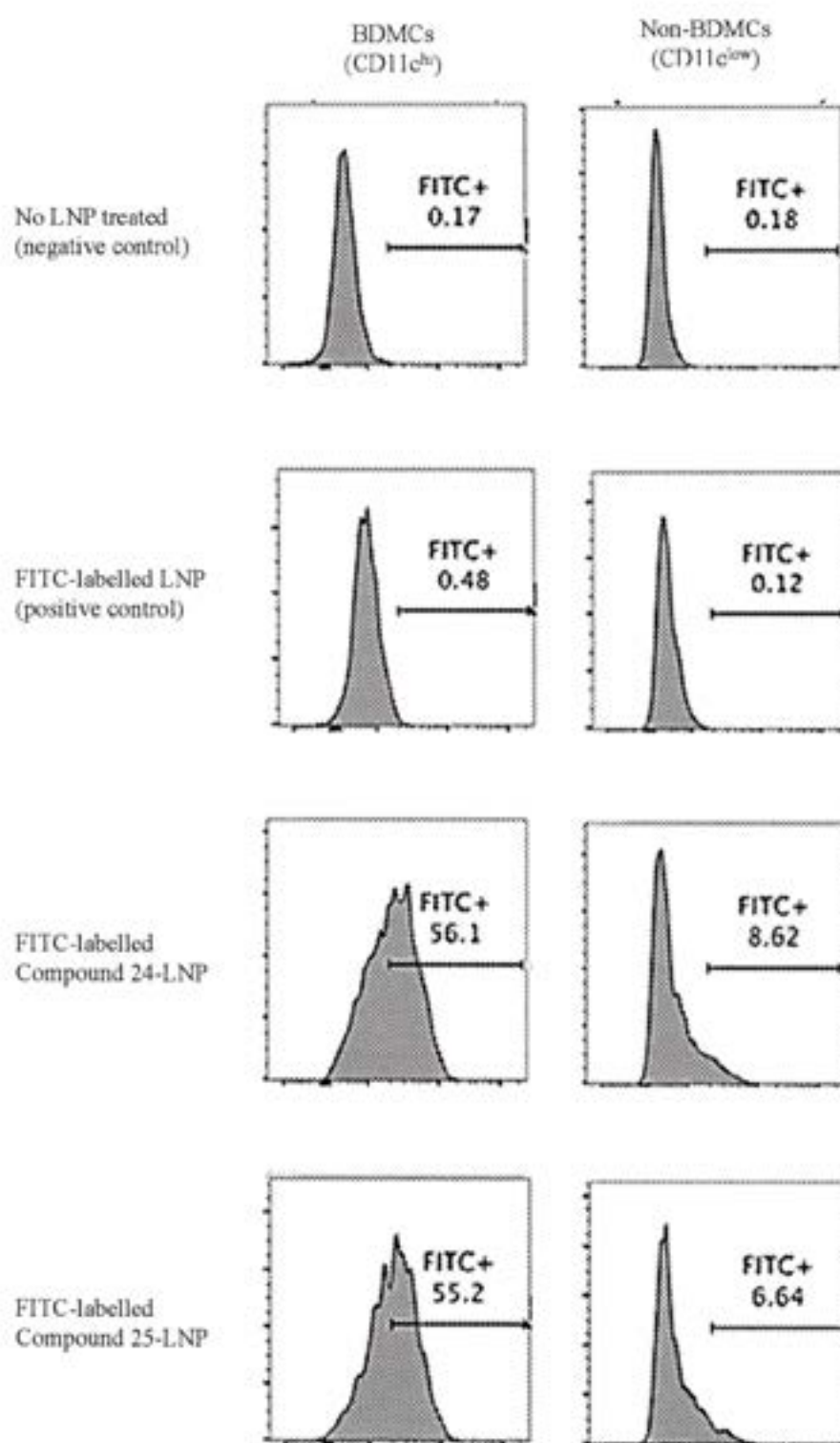


FIG. 1

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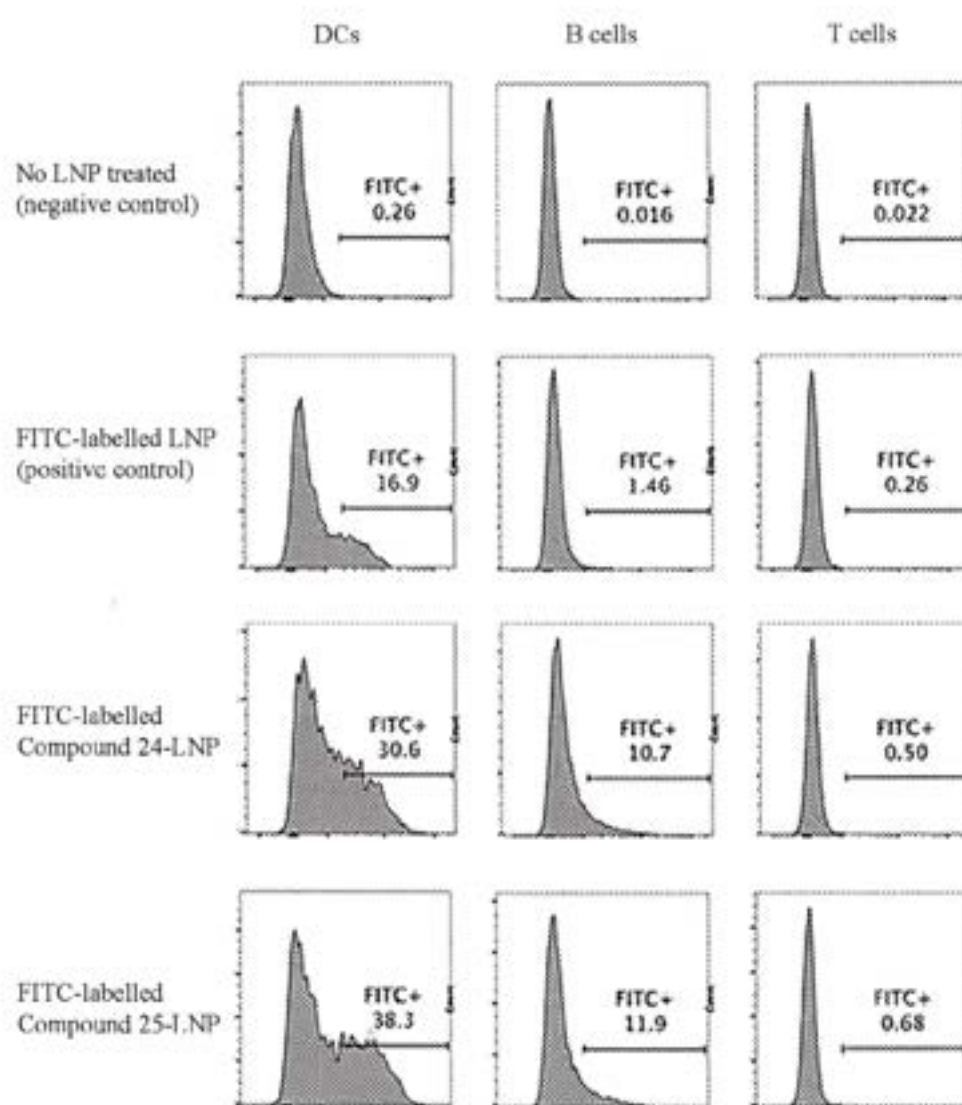


FIG. 2

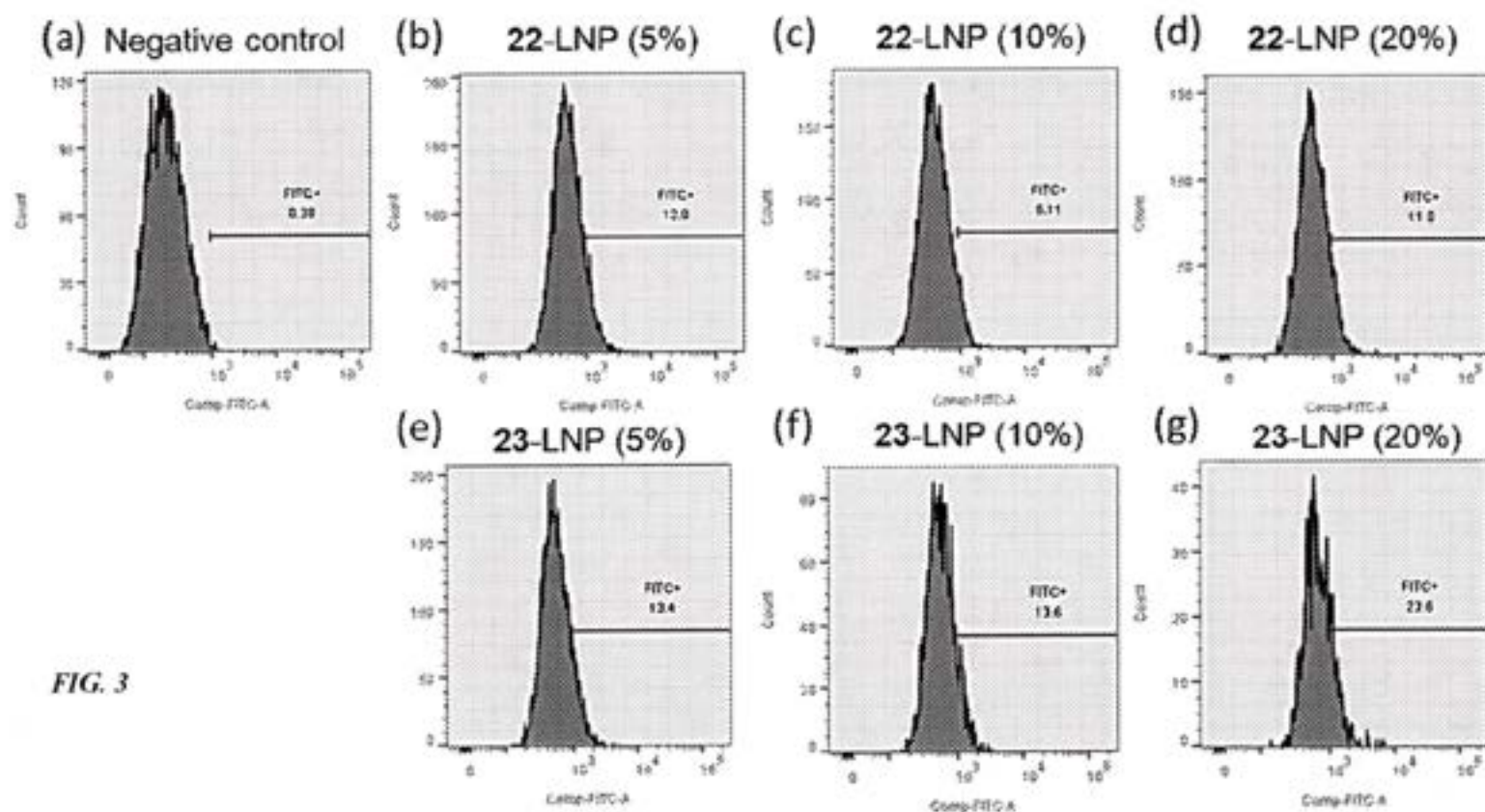


FIG. 3

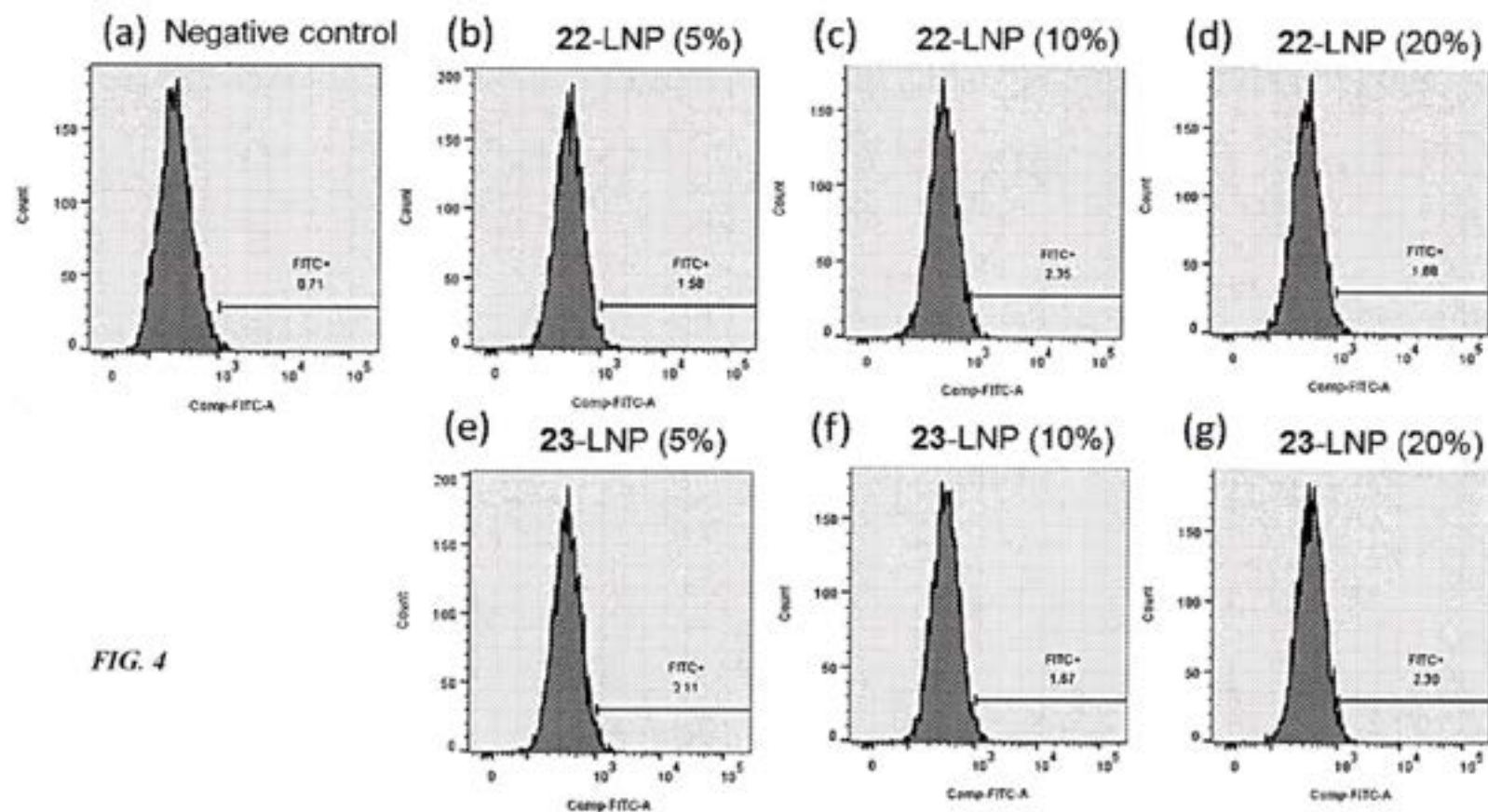


FIG. 4

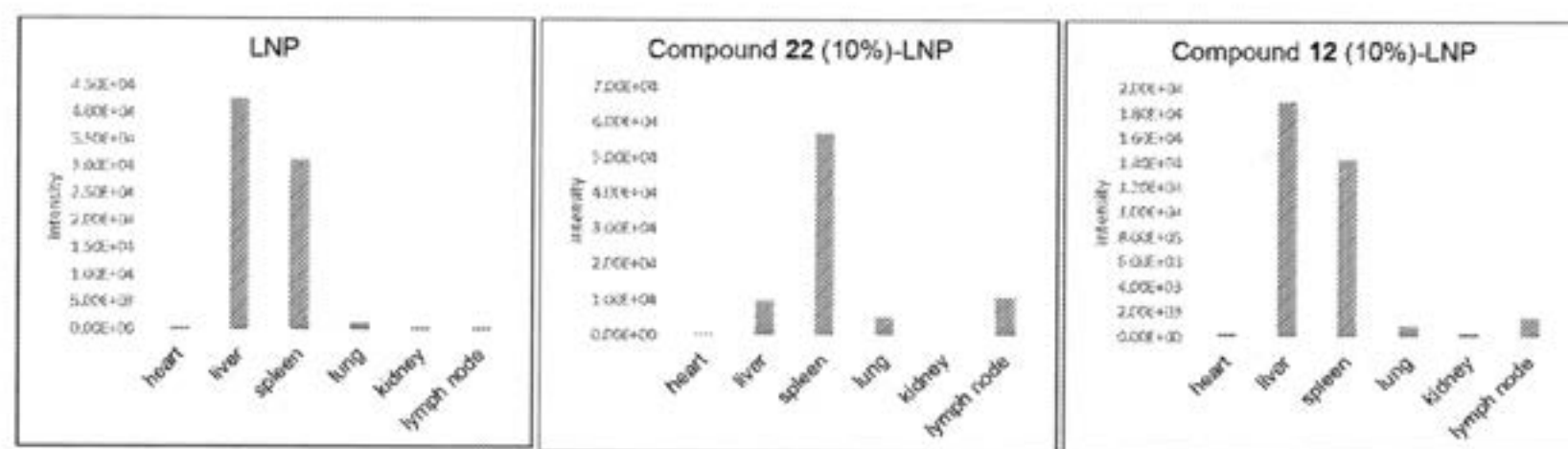


FIG. 5

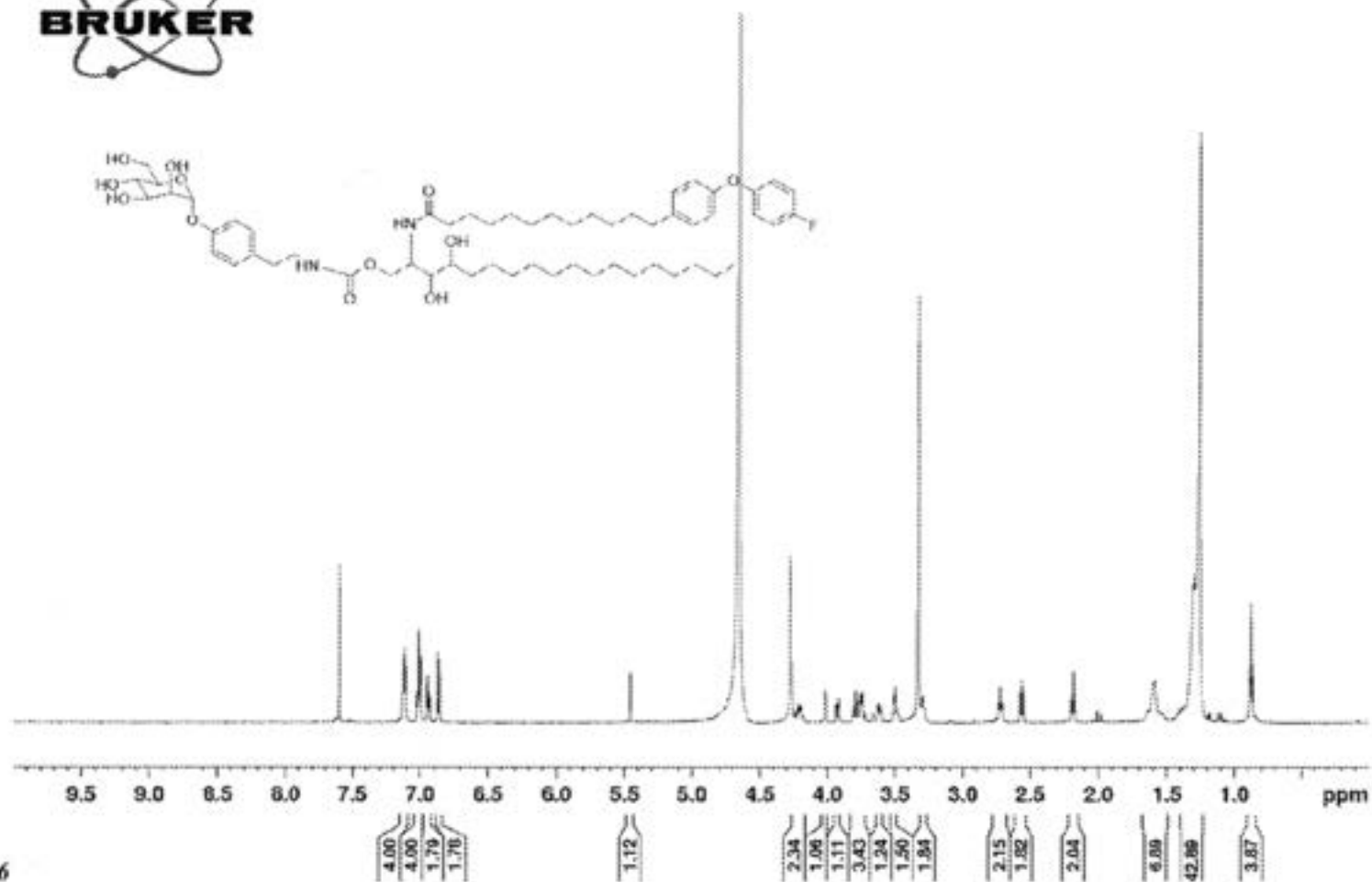
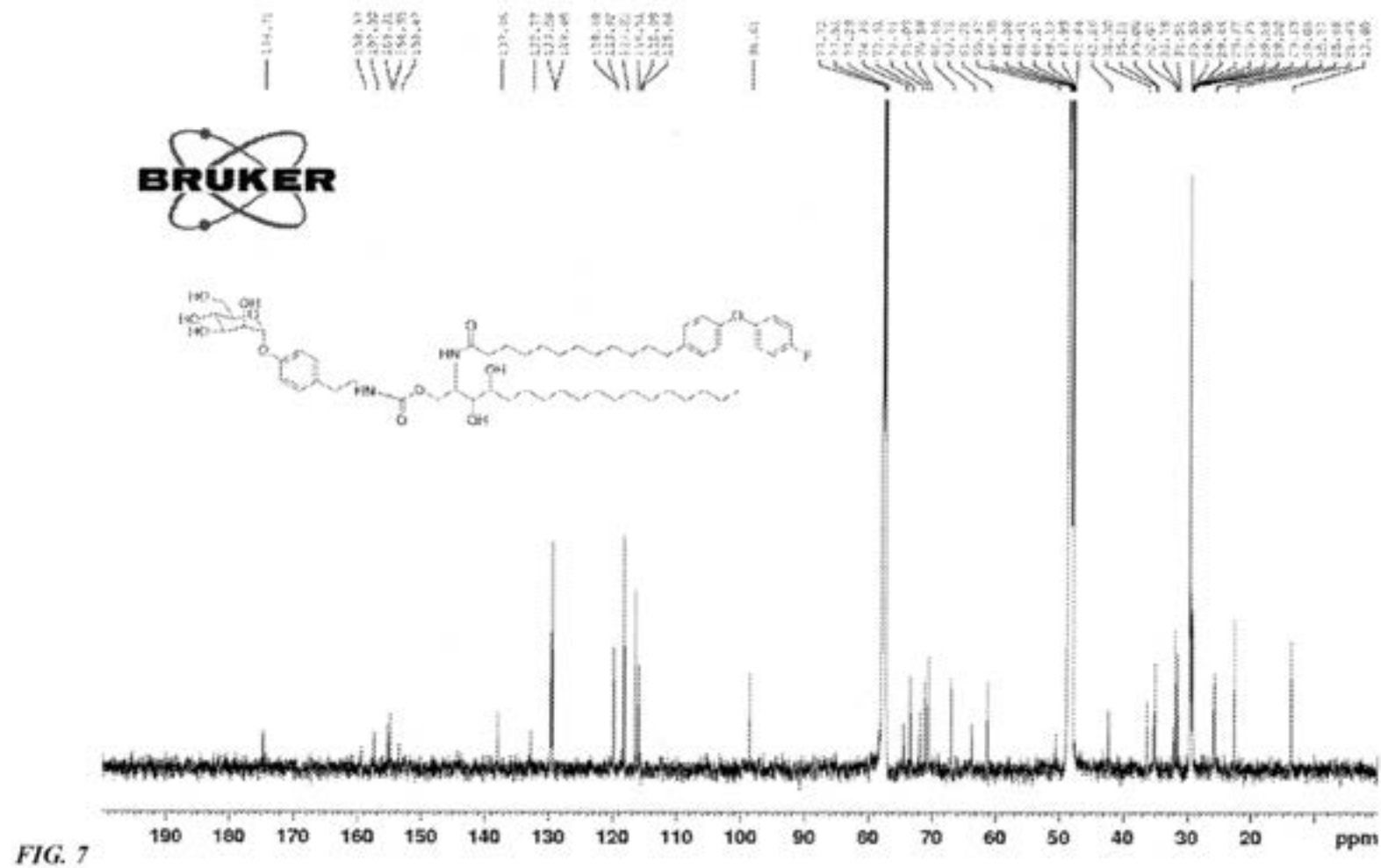
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FIG. 6

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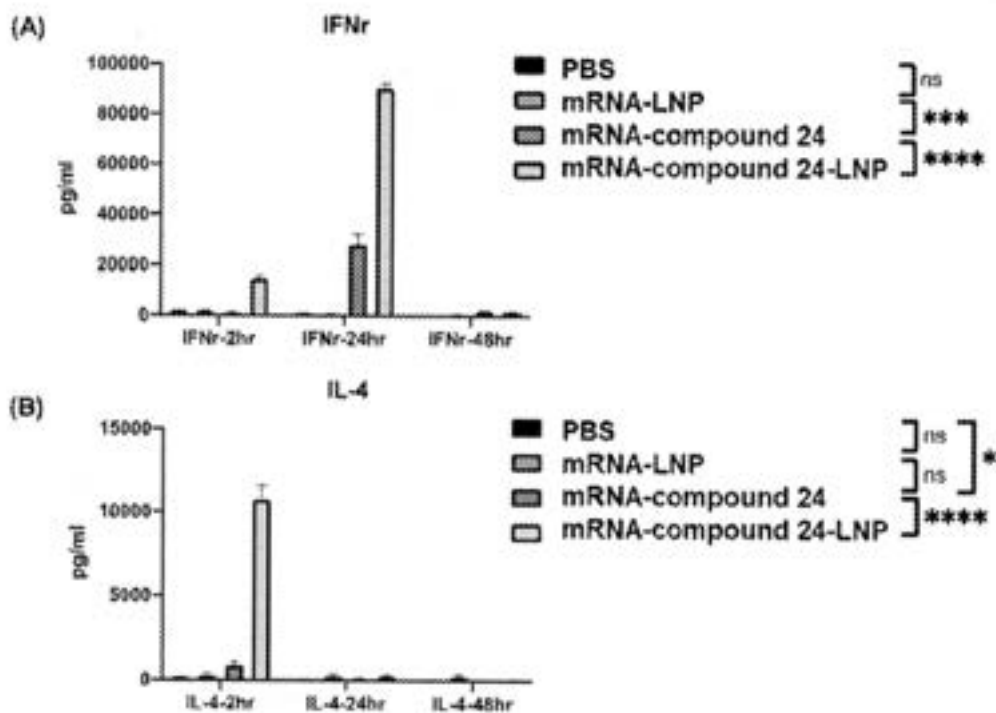


FIG. 8

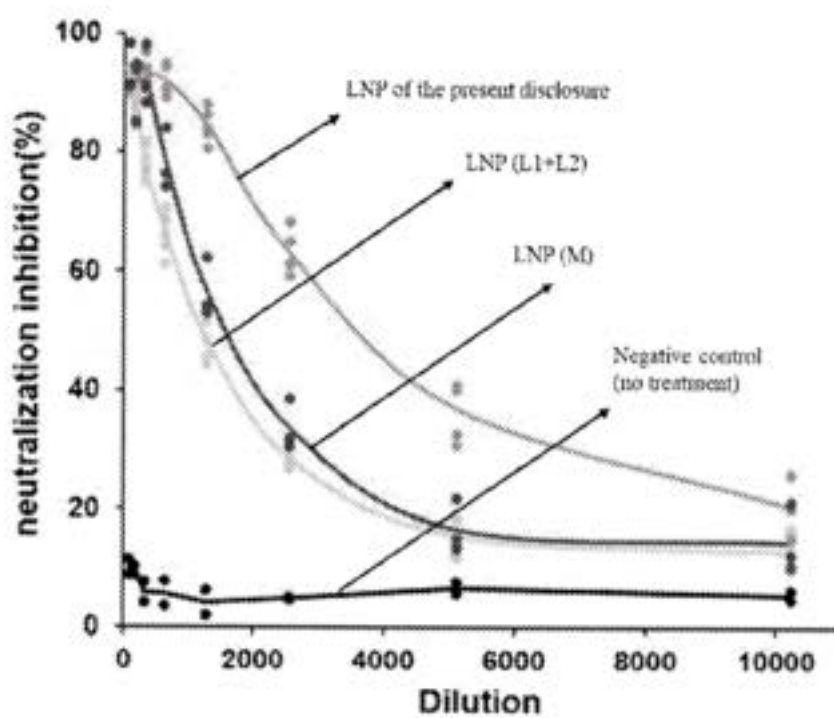


FIG. 9

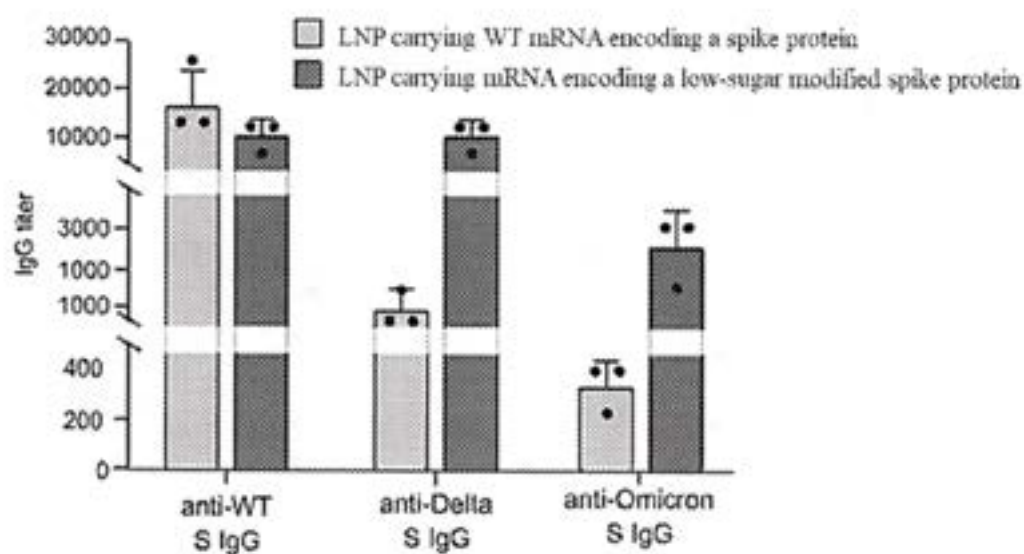


FIG. 10

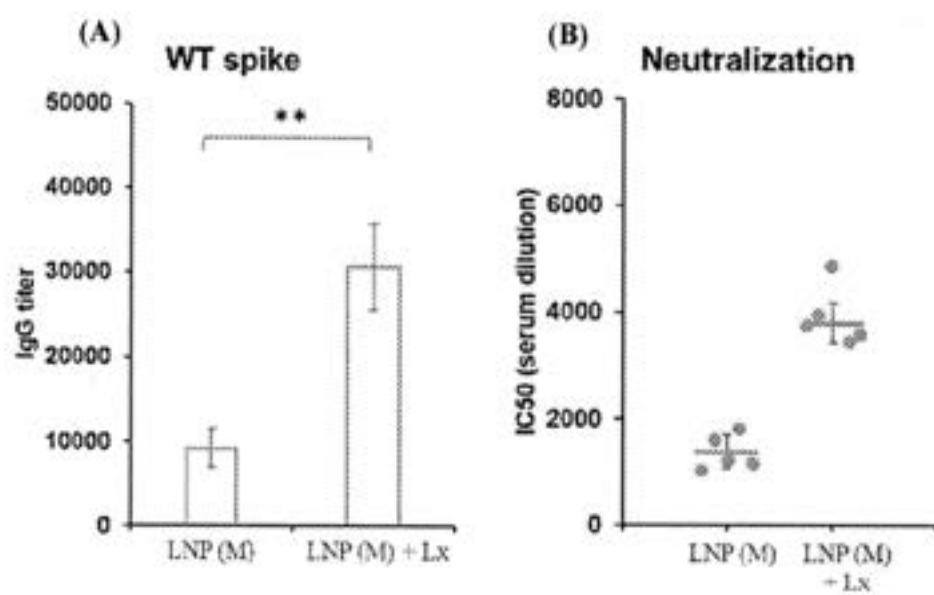


FIG. 11

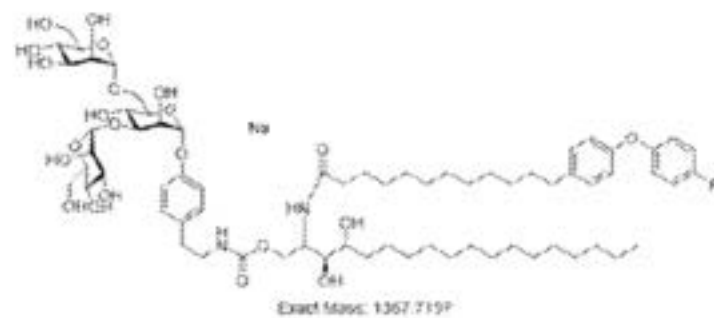
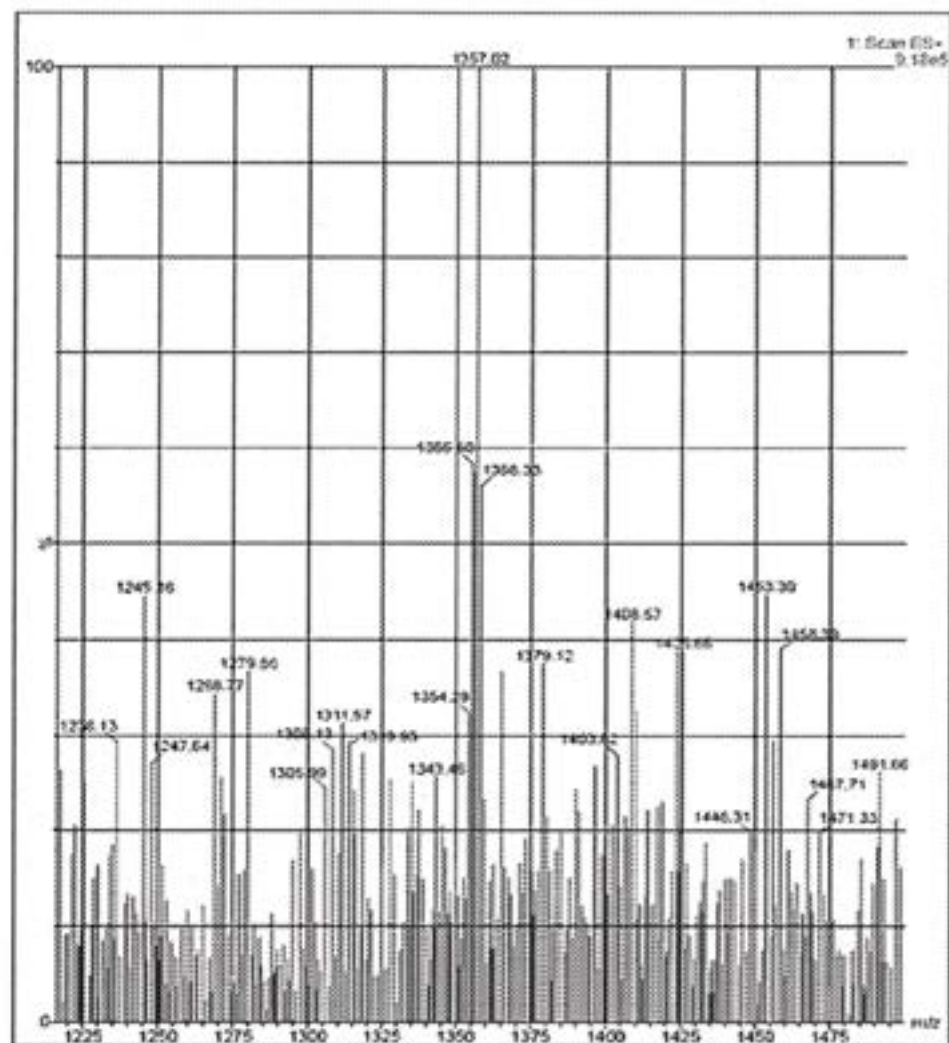
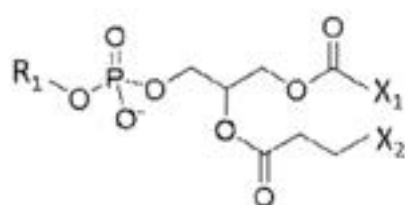


FIG. 12



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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.

[0004] 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.

[0005] 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.

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] 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.

[0011] 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

[0012] 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.

[0013] FIG. 2 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 uptake of the analysis demonstrating the efficacy 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.

[0014] 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. 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).

[0015] 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 according to embodiments of the present disclosure targeting BDMCs compared with LNPs without the compound of the present disclosure. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units. 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. 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 tested animals. The assay generates 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.

[0017] FIG. 6 shows the ^1H NMR spectrum of compound 12 of the present disclosure.

[0018] FIG. 7 shows the ^{13}C NMR spectrum of compound 12 of the present disclosure.

[0019] FIG. 8 presents bar charts showing the IFN γ (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.

[0020] 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 using different dilution factors to show the difference between samples.

[0021] 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.

[0022] 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.

[0023] FIG. 12 shows the LCMS spectrum of compound 21 of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

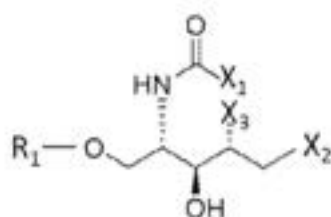
[0024] Nanoparticles have been widely used in various applications. Among them, **lipid nanoparticles (e.g., liposomes)** are the most well-established delivery systems for medicines due to their biocompatibility and biodegradability. A typical liposome has a bilayer structure formed by phospholipids 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.

[0025] 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.

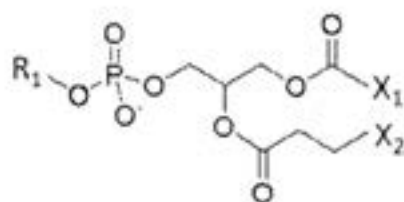
[0026] **TARGETING LIPID COMPOUND**

[0027] 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 structural moiety, which can be incorporated into a lipid bi-layer such as an LNP. 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 compound, 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.

[0028] 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.

[0029] *R₁ group*

[0030] *Targeting Functionality.* In certain embodiments, the R_1 group is configured to provide selective 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 receptor on a target cell. For example, the R_1 group might be configured to target the DC-SIGN of a dendritic cell.

[0031] 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.

[0032] 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- α 2,3-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-2, and the glycosyl group can comprise 9-Biphenyl Neu5Ac- α 2,6-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-5/E, and the glycosyl group can comprise Neu5Ac- α 2,3-Gal-GlcNAc.

[0033] In some embodiments, the R_1 group 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 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, wherein the substituent of the aryl is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide. 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.

[0034] *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.

[0035] *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.

[0036] 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.

[0037] *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.

[0038] 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_{1-6} 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. 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).

[0039] 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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.

[0040] 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof. For example, the substituted benzene ring can be a phenol group.

[0041] In some embodiments, the R₁ group is a mono-mannoside substituted with 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof. In certain embodiments, the R₁ 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 C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, and sulfite.

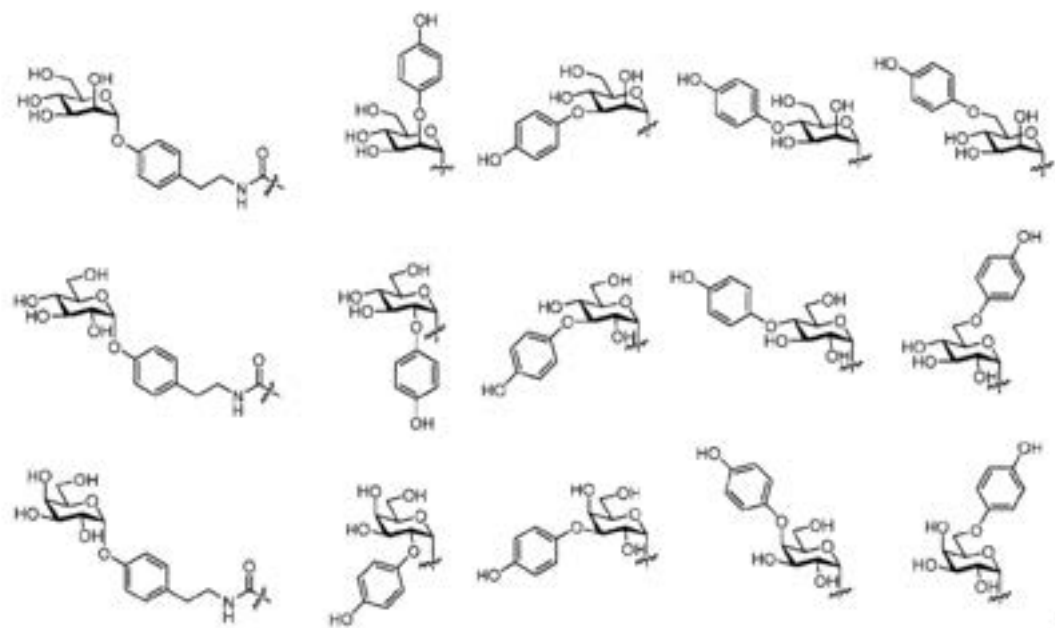
[0042] In some embodiments, the R₁ group comprises a first mannoside and a second mannoside. Each of the first mannoside and the second mannoside is independently substituted with 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

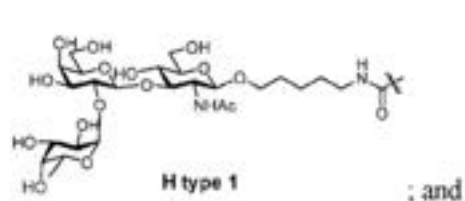
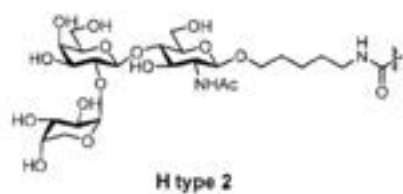
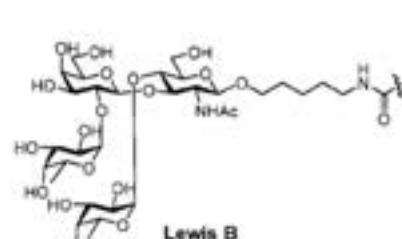
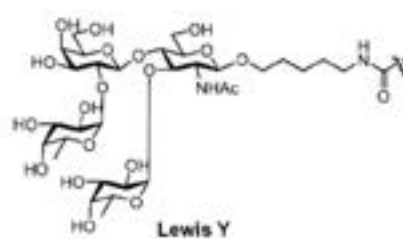
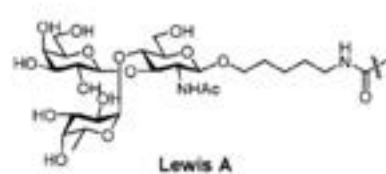
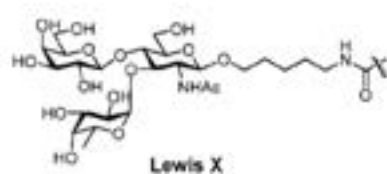
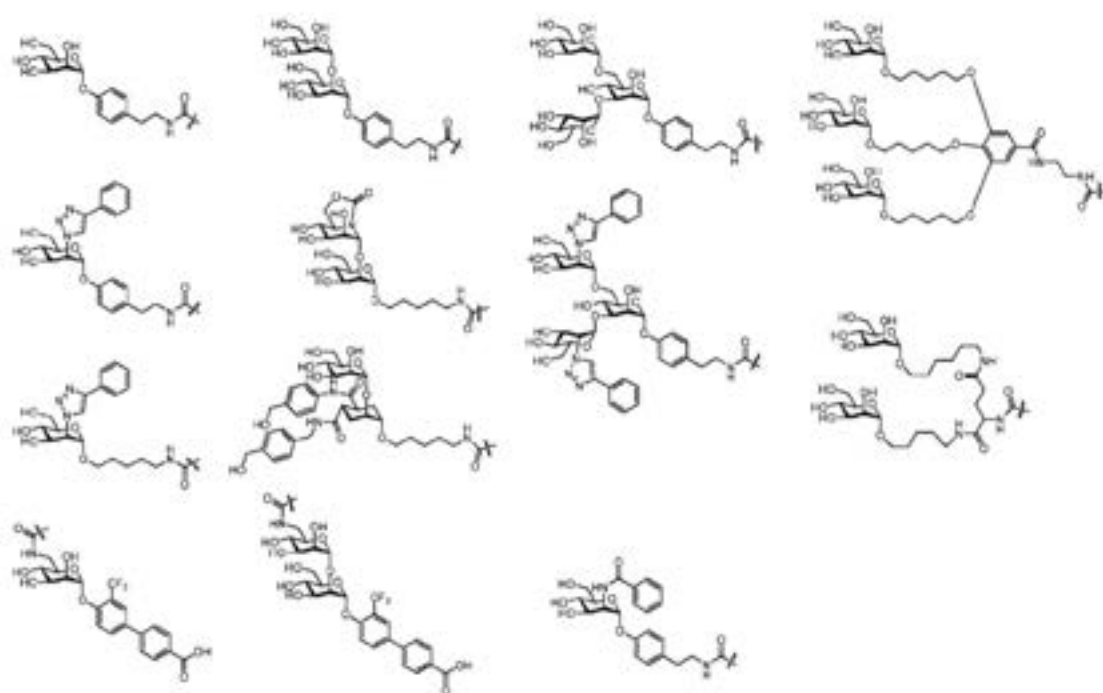
[0043] *Binding affinity.* In some embodiments, the binding affinity between the glycosyl group of R₁ and a target can be defined by a dissociation constant (K_D). In some embodiments, the K_D at pH 7.4 can be 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 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

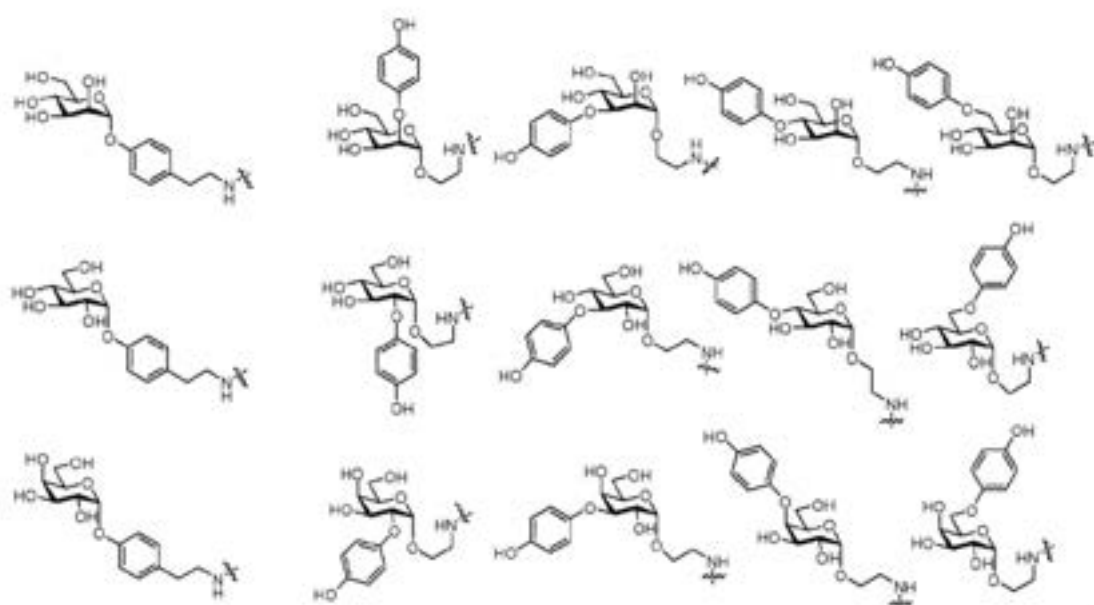
300, 5 to 200, 5 to 150, 5 to 100, 5 to 75, 5 to 50, 5 to 30, 5 to 20, 10 to 8000, 10 to 7000, 10 to 6000, 10 to 5000, 10 to 4000, 10 to 3000, 10 to 2500, 10 to 2000, 10 to 1500, 10 to 1250, 10 to 1000, 10 to 900, 10 to 800, 10 to 700, 10 to 600, 10 to 500, 10 to 400, 10 to 300, 10 to 200, 10 to 150, 10 to 100, 10 to 75, 10 to 50, 10 to 30, or 10 to 20 nM.

[0044] 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.

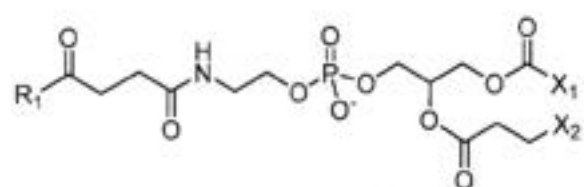
[0045] *Examples.* In some embodiments, the R_1 group is selected from the group consisting of:





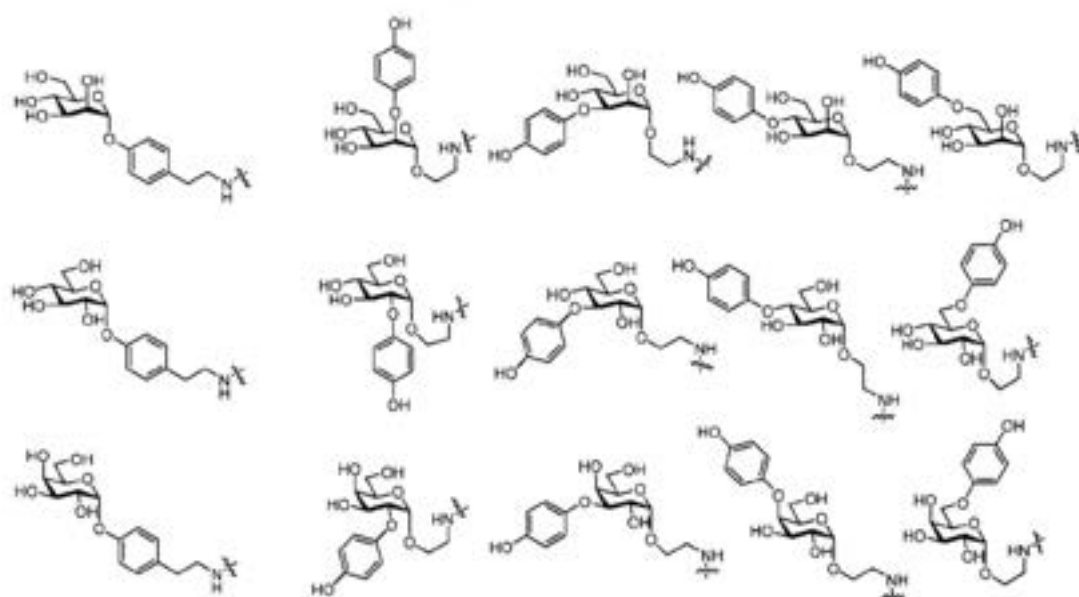


[0046] In some embodiments, the compound of the present disclosure has the structure shown in Formula 3:



Formula 3; and

wherein the R₁ group is selected from the group consisting of:



[0047] X_1 and X_2

[0048] 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.

[0049] 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.

[0050] 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.

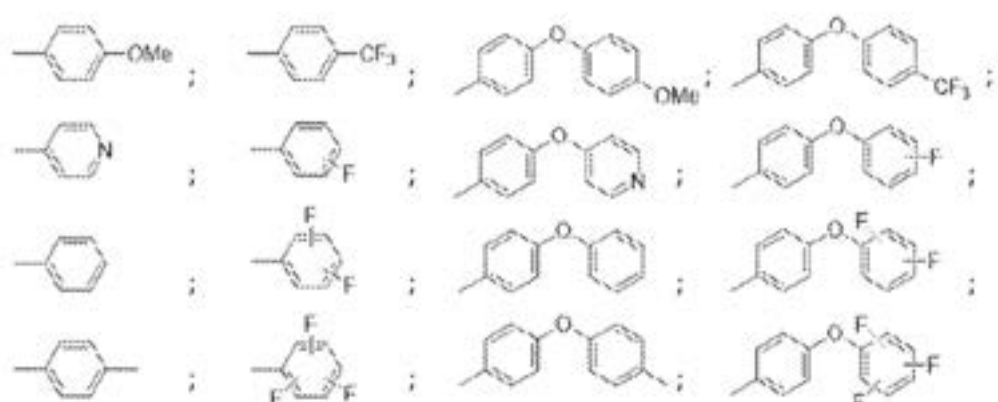
[0051] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{8-30} alkyl, C_{8-30} alkenyl, C_{8-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)_nX_4$, n is 8 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided 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.

[0052] 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.

[0053] 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 .

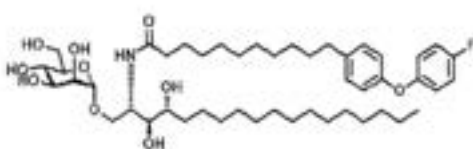
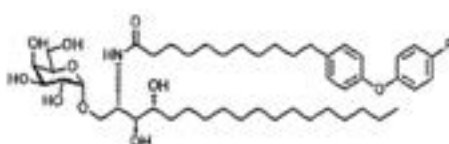
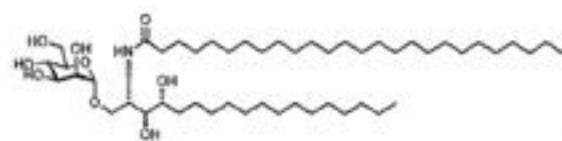
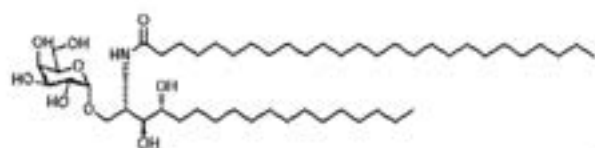
[0054] In some embodiments, X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

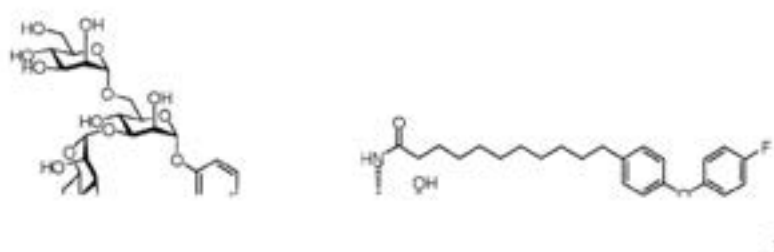
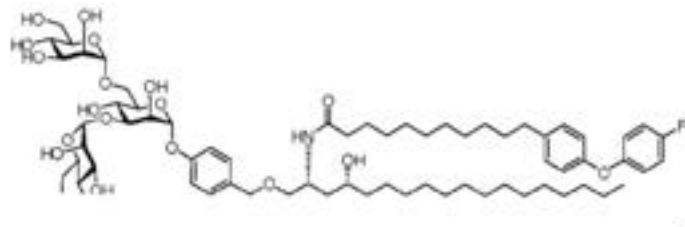
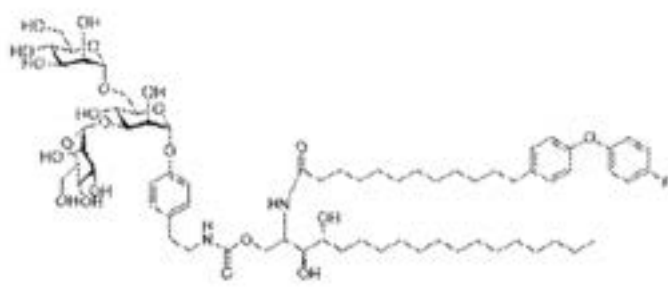
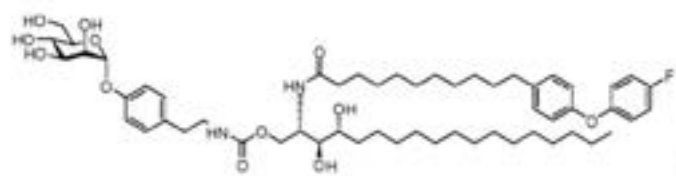
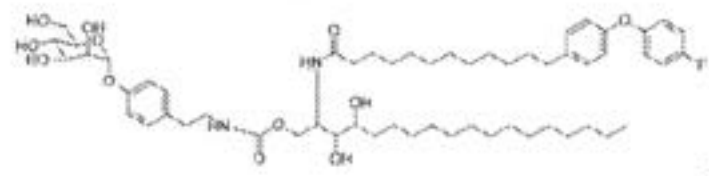
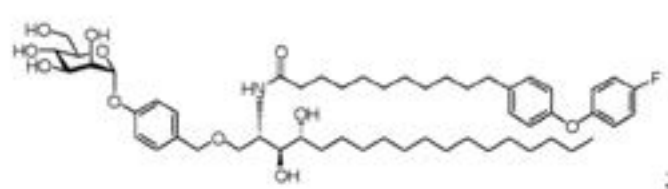
[0055] In certain embodiments, X_4 is selected from the group consisting of:

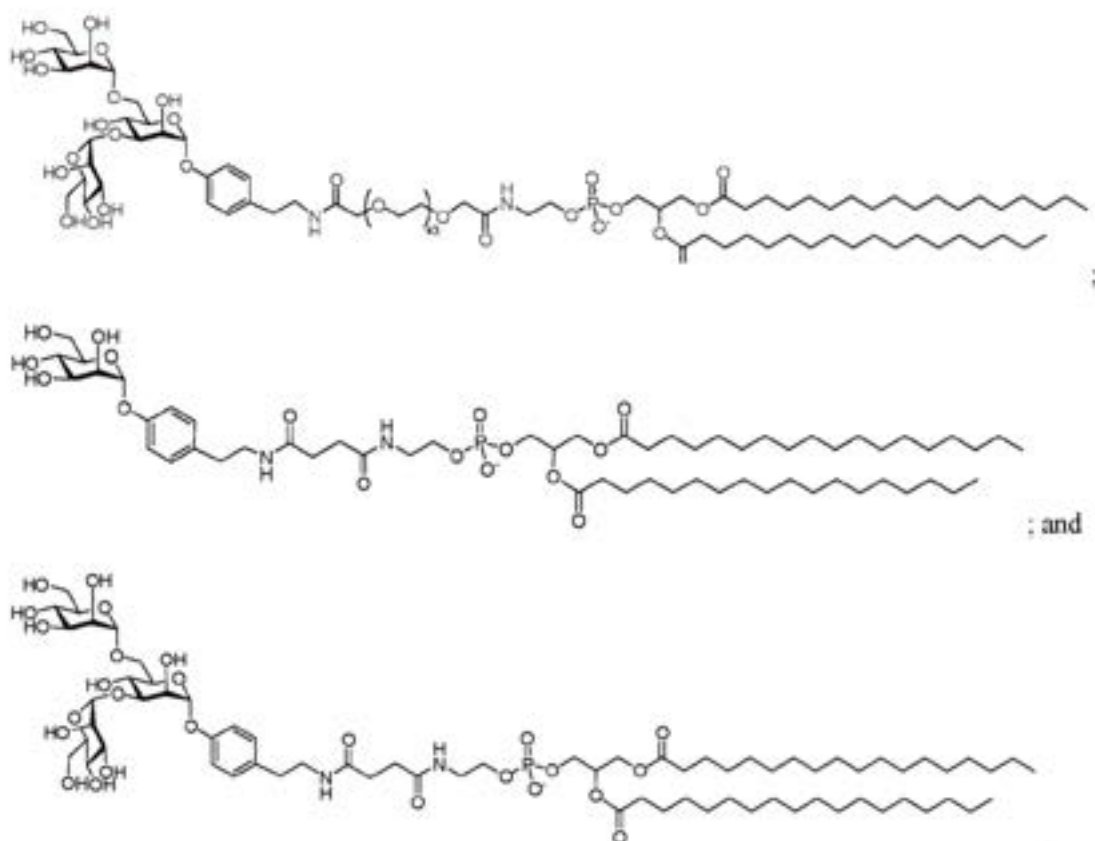


[0056] **Exemplary Compound of the Present Disclosure**

[0057] 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).







[0058] COMPOSITION FOR FORMING A LIPID NANOPARTICLE

[0059] 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.

[0060] 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 R₁ group targeting the same target, or different targeting moiety of the R₁ group targeting different targets.

[0061] 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.

[0062] 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.

[0063] ***Ionizable lipid.***

[0064] 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-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediyldis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0065] ***Helper lipid.***

[0066] A helper lipid is a lipid used to increase particle stability and fluidity of lipid nanoparticles. A helper 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.

[0067] 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.

[0068] *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.

[0069] *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, (3 β ,5 β)-3-hydroxycholestan-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.

[0070] *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 R₁ comprises a PEG moiety. In those circumstances, the composition does not need to comprise a PEG-lipid.

[0071] *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.

[0072] 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%

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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%

[0073] **LIPID NANOPARTICLE (LNP)**

[0074] 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%.

[0075] 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.

[0076] 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%.

[0077] *Size of the LNP.*

[0078] 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.

[0079] *Zeta potential and molecular weight.*

[0080] Without wishing to be bound by theories, the zeta potential and molecular weight of a polymersome may affect the cellular uptake of the polymersome. In some embodiments, the polymersome 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 polymersome 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.

[0081] *Payload*

[0082] 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.

[0083] 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.

[0084] In some embodiments, the polypeptide or the protein is immunogenic (e.g., antigenic) to an organism to which the polymersome is administered. In such embodiments, the polymersome 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., 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.

[0085] 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'-O 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.

[0086] 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).

[0087] 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.

[0088] 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.

[0089] 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.

[0090] In some embodiments, the N/P ratio (positively-chargeable polymer amine (N = nitrogen) groups to negatively-charged nucleic acid phosphate (P) groups) of the polymersome 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, a polymersome encapsulating a mRNA has a nanoparticle/mRNA (N/P) ratio of about 10 or about 20.

[0091] 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*.

[0092] *Kit for Preparing a Lipid Nanoparticle.*

[0093] 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.

[0094] *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.

[0095] **COMPOSITIONS/FORMULATIONS**

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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).

[0101] 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.

[0102] *Combination compositions*

[0103] 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.

[0104] 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.

[0105] 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.

[0106] *Additional components of the composition*

[0107] 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.

[0108] 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.).

[0109] 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.

[0110] 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).

[0111] 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).

[0112] 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.

[0113] **METHODS OF USE**

[0114] 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.

[0115] *Methods of targeted payload delivery in a subject*

[0116] 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.

[0117] *Methods of preventing or treating a disease in a subject*

[0118] 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.

[0119] 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 R₁ 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 R₁ 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.

[0120] 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.

[0121] 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.

[0122] *Methods of boosting an adaptive immune response*

[0123] 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.

[0124] 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.

[0125] 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.

[0126] *Administration*

[0127] 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.

[0128] *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.

[0129] *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.

[0130] 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).

[0131] 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.

[0132] 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.

[0133] 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%.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] **DEFINITION**

[0139] 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.

[0140] 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 $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate.

[0141] 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.

[0142] 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.

[0143] 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.

[0144] 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.).

[0145] 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.

[0146] 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, C₂₋₆ 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.

[0147] 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, C₂₋₆ 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.

[0148] As used herein, "cycloalkyl" refers to a nonaromatic cyclic, bicyclic, 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.

[0149] 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.

[0150] 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, quinoliny, isoquinoliny, cinnoliny, phthalazinyl, quinazolinyl, quinoxalinyl, triazinyl, indolyl, isoindolyl, indazolyl, indoliziny, purinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, benzoxazolyl, benzothiazolyl, 1H-benzimidazolyl, imidazopyridinyl, benzothienyl, benzofuranyl, isobenzofuran and the like.

[0151] 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 —NH₂, —NHR, or —NR₂, where R is the same or different at each occurrence and can be an alkyl group or an aryl group.

[0152] 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.

[0153] 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. Dihalalkyl 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.

[0154] As used herein, unless otherwise specified, the term “heteroatom” refers to a nitrogen (N), oxygen (O), or sulfur (S) atom.

[0155] **EXAMPLE**

[0156] **Example 1: Synthesis of exemplary compounds of the present disclosure**

[0157] *Chemical Materials and Methods*

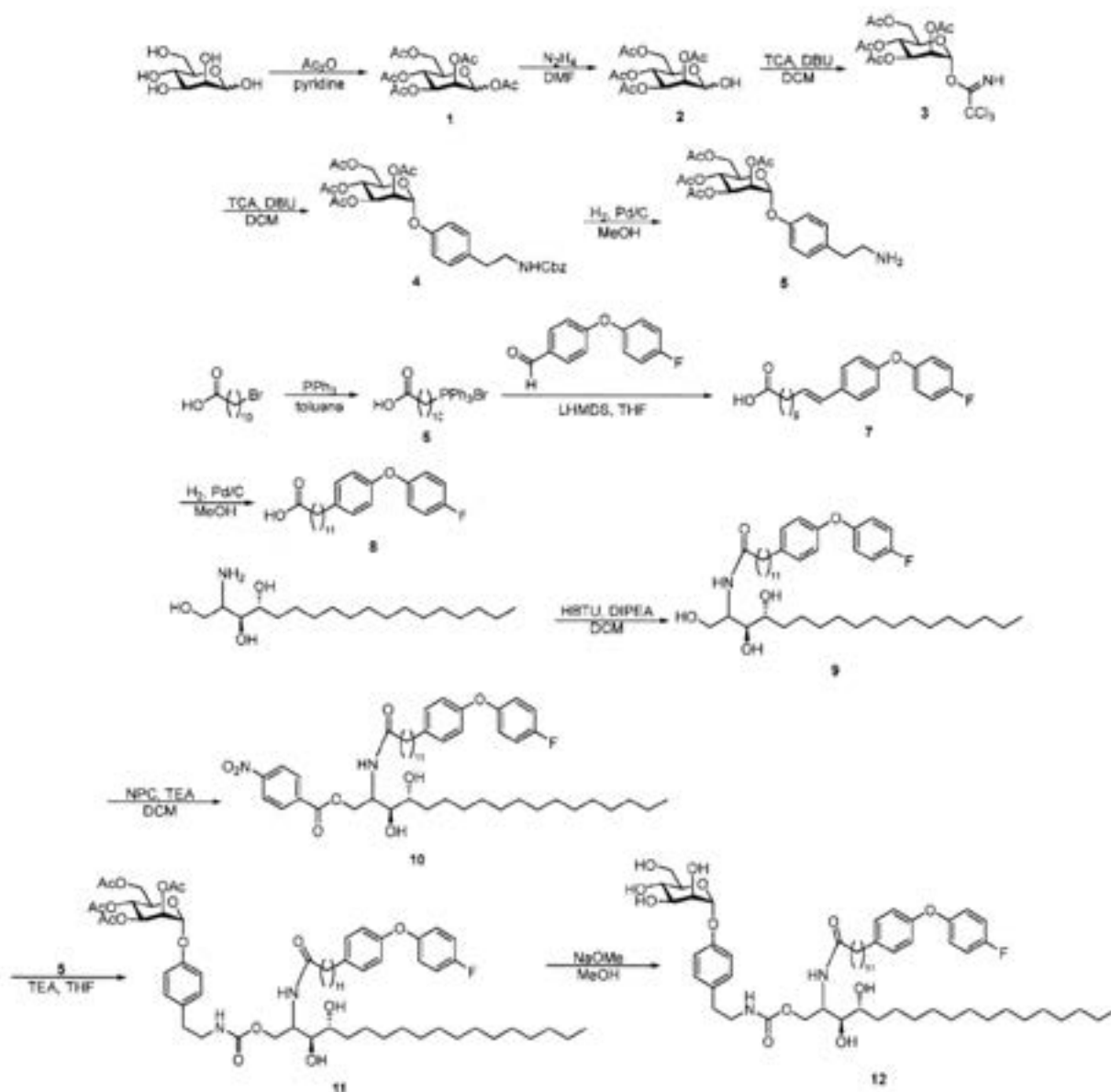
[0158] For chemical synthesis, all starting materials and commercially obtained reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry solvents. ^1H and ^{13}C NMR spectra were recorded on Bruker 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 $\text{DMSO}-d_6$ at δ 2.5 and 39.51 for ^1H 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. IMPACTTM 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.

[0159] The chemical materials and methods described herein apply to all examples described in the present disclosure.

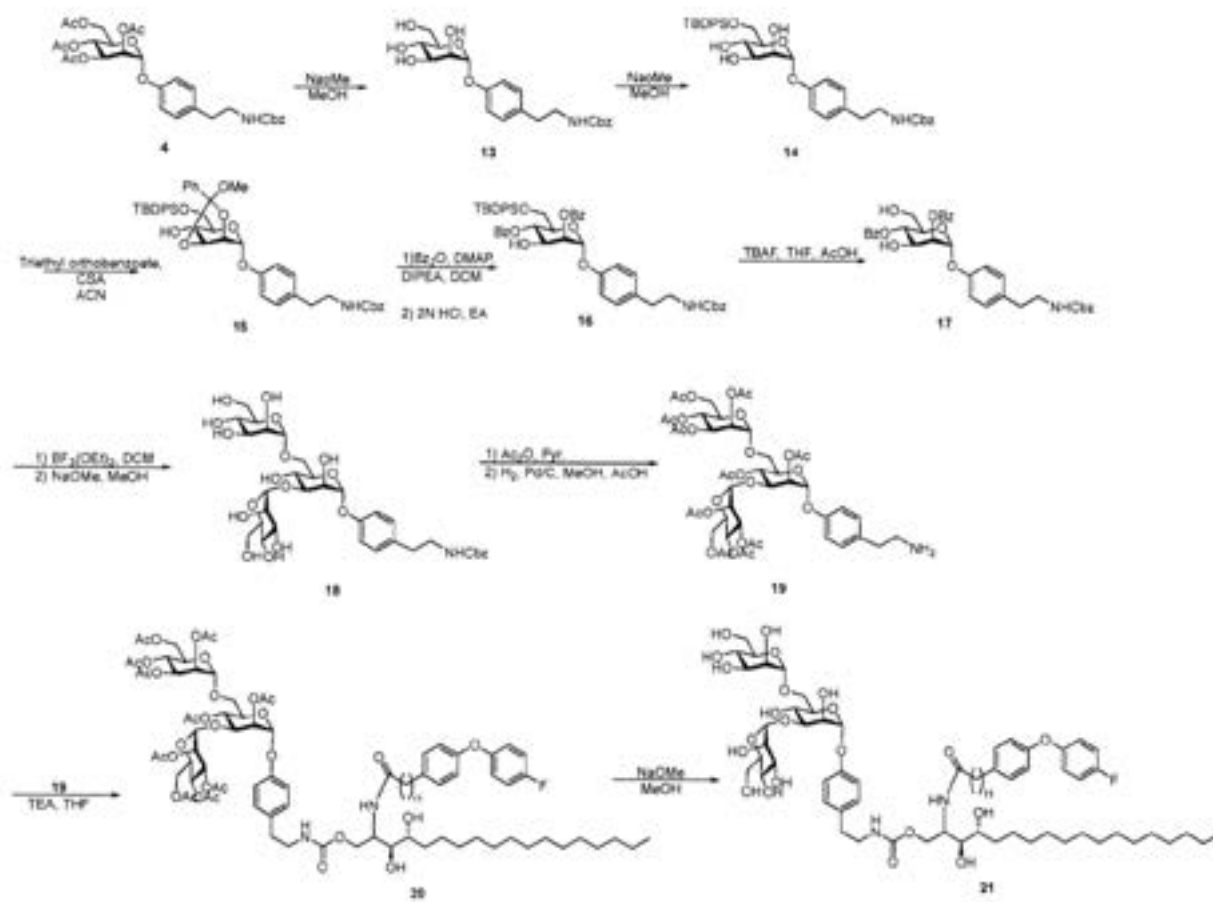
[0160] *Synthesis and Results*

[0161] 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.

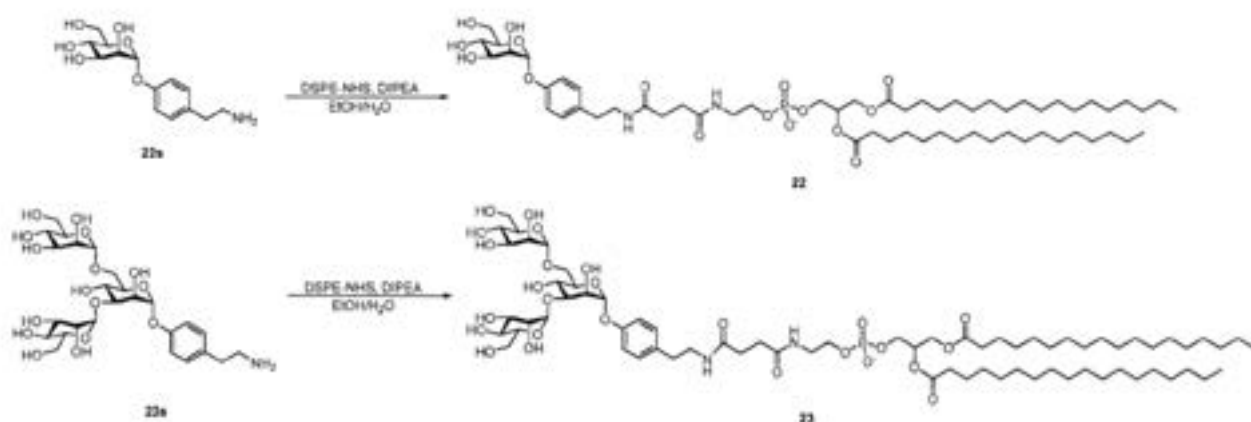
[0162] Scheme 1



[0163] Scheme 2



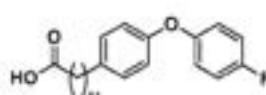
[0164] Scheme 3



[0165] Compounds 1 to 5

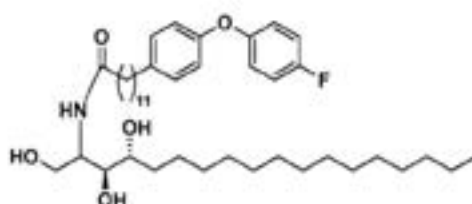
[0166] Compounds 1-5 were synthesized and characterized according to a published protocol (ACS Nano 2021, 15, 309–321).

[0167] Compound 8



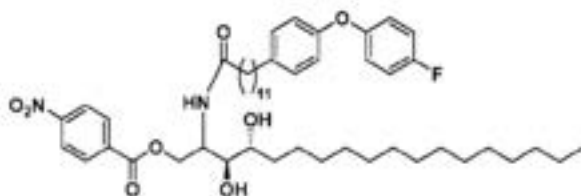
[0168] (11-Carboxynonyl)triphenylphosphonium bromide **6** (2.5 g, 10 mmol) was prepared by refluxing triphenylphosphine (10 mmol) and 11-bromoundecanoic acid (10 mmol). It was then dissolved in 50 ml of tetrahydrofuran (THF) and cooled to 0 °C. lithium bis(trimethylsilyl)amide (LHMDS; 1 M in THF, 20 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 MgSO₄. 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 H₂ 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%).

[0169] Compound 9



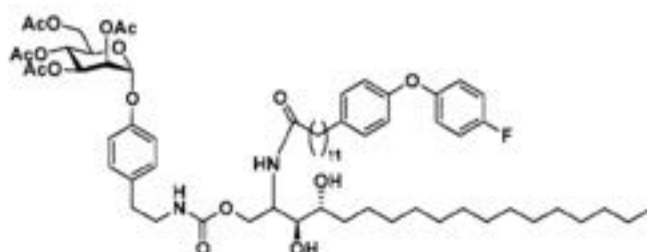
[0170] Compound 9. Compound 8 (1 mmol) in THF (10 mL) was added EDC (1.5 mmol), HOBt (1.5 mmol), DMAP (0.1 mmol), trimethylamine (2 mmol), and phytosphingosine (1.2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1) to yield 9 (74%).

[0171] Compound 10



[0172] 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.

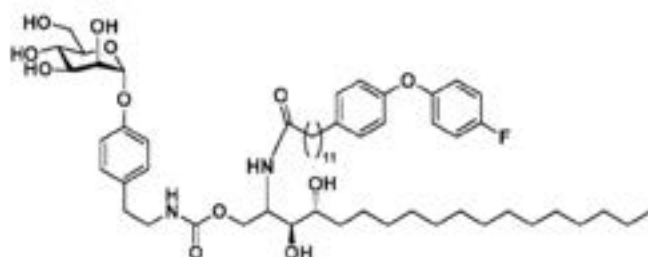
[0173] Compound 11



[0174] 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/H₂O. The collected organic layer was washed with

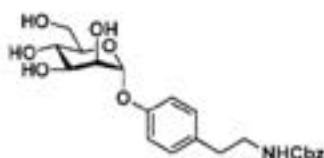
saturated $\text{NaHCO}_3(\text{aq})$, water and brine, and dried over MgSO_4 . The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **11** (59%).

[0175] **Compound 12**



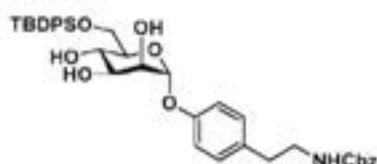
[0176] Compound **11** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at room temperature for 2 hours. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **12** (quant.).

[0177] **Compound 13**



[0178] 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.).

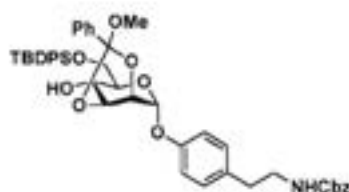
[0179] **Compound 14**



[0180] 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

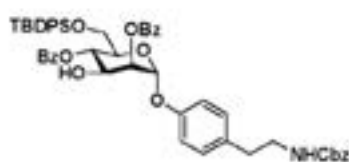
MeOH. After stirring at room temperature for 10 min, the solvent was removed under reduced pressure to give a dry residue that was purified by column chromatography with MeOH/DCM (1/10) to give compound **14** (82%).

[0181] **Compound 15**



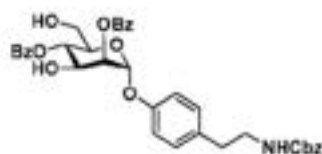
[0182] To a solution of compound **14** (1 mmol) and a catalytic amount of CSA (0.1 mmol) in CH₃CN (20 mL) was added trimethyl orthobenzoate (3 mmol) at room temperature under atmospheric pressure of nitrogen. After stirring for 30 min, Et₃N was added to quench the reaction, and the resulting mixture was dried under reduced pressure. The residue was purified by column chromatography with EA/Hex (1/2) to give compound **15** (79%).

[0183] **Compound 16**



[0184] 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/H₂O. The collected organic layer was washed with ice-cold saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **16** (71%).

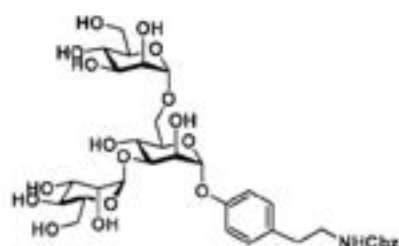
[0185] **Compound 17**



[0186] 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 NaHCO₃(aq), water, and brine, dried with anhydrous MgSO₄, and concentrated under reduced pressure. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **47** (88%).

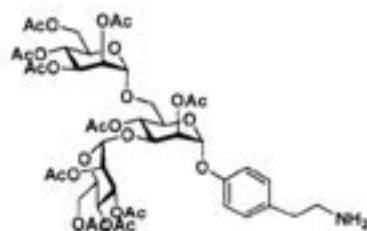
[0187]

[0188] **Compound 18**



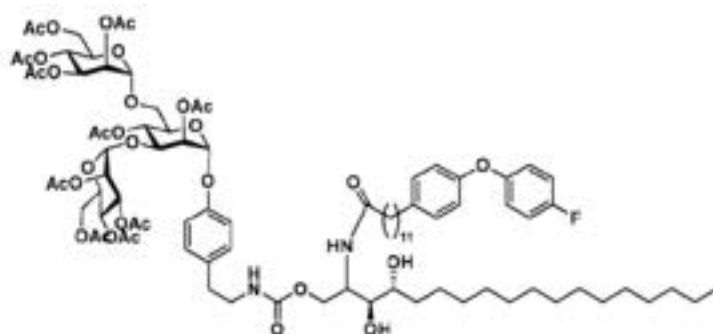
[0189] To a stirred solution of **17** (1 mmol) and 4 Å molecular sieve (0.1 g) in anhydrous DCM (10 mL) was cooled to -40°C and then BF₃(OEt)₂ (0.1 mmol) was added dropwise to the solution. A solution of **3** in anhydrous DCM was added dropwise to the above mixture and stirred for 1 h at -40°C. After 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 NaHCO₃ aq. and extracted with DCM. The organic layer was dried with MgSO₄ 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 H₂O as eluent to obtain a pure trisaccharide. The compound was lyophilized to dryness to give compound **18** (39%).

[0190] **Compound 19**



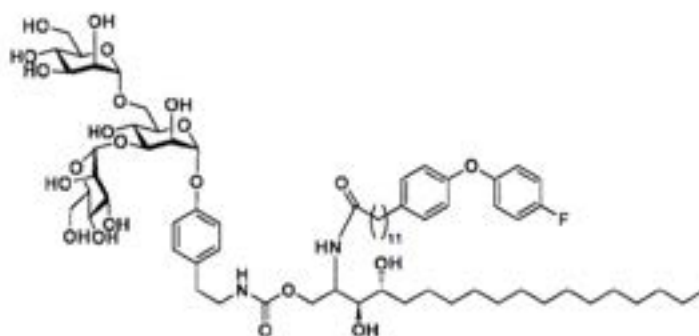
[0191] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo.

[0192] **Compound 20**



[0193] 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/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **20**.

[0194] **Compound 21**



[0195] 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).

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.

[0203] *Characterization of LNP*

[0204] *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.

[0205] 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

[0206] *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.

[0207] Table: Zeta potential and encapsulation efficiency

Sample	Zeta potential (mV)	Encapsulation efficiency (%)
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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

[0208] **Example 3: *In vitro* uptake and transfection of mRNA-LNPs in Dendritic cells.**

[0209] *Experiment 3-1*

[0210] *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 μ M 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 μ M 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.

[0211] 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

[0212] *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 °C 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).

[0213] *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 (BioLegend) 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).

[0214] *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-BMDCs, 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.

[0215] 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

[0216] *Experiment 3-2*

[0217] 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, 1×10^6 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.

[0218] 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°C for 48 hours. The experiments were conducted using FACS, similar to that described above.

[0219] 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

[0220] *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.

[0221] 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

[0222] *Experiment 3-3*

[0223] 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 CaCl₂, 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 tetramethylbenzidine (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 K_d was calculated using a nonlinear regression curve fit for total binding using GraphPad Prism.

[0224] **Example 4: *In vivo* delivery of luciferase mRNA-LNP**

[0225] 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 CO₂ 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.

[0226] 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

[0227] *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.

[0228] **Example 6: Immunization**

[0229] *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).

[0230] *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.

[0231] *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.

[0232] *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 AlamarBlue 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 titrating 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 $(RLU^{control} - RLU^{serum})/RLU^{control}$.

[0233] *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 H₂SO₄. After incubation for 30 minutes, absorbance (OD 450 nm) was measured using SpectraMax M5.

[0234] *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.

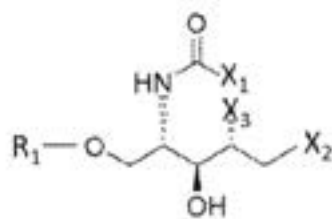
[0235] FIG. 10 verifies that the LNPs of the present disclosure were capable of inducing antigen-specific IgG *in vivo*. The LNP, carrying mRNA encoding wide-type spike protein, induced IgGs that were still able to recognize the spike proteins of both the Delta variant and Omicron variant at a good level. 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.

[0236] 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

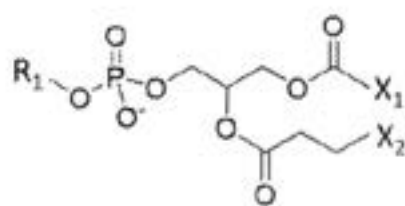
compound of the present disclosure improves the performance of the Moderna formulation. Animals were administered with the LNPs, and IgG titer assay and neutralization assay were all performed as described above in this example. The results demonstrate that the compound of the present disclosure increased 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.

What is claimed is:

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;

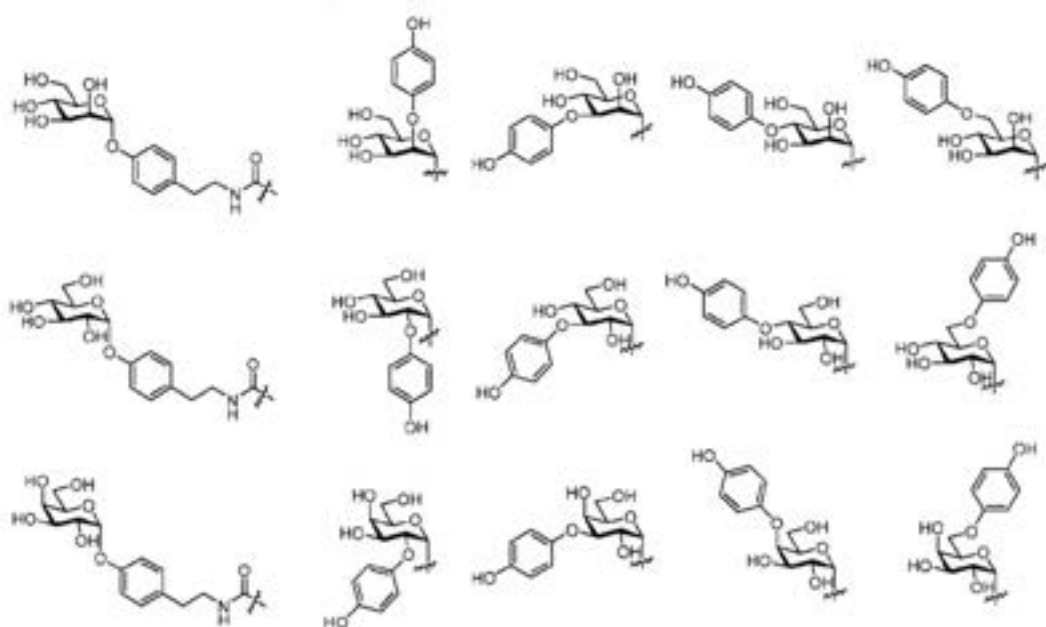
wherein X_1 and X_2 are each independently hydrogen, C_{1-30} alkyl, C_{1-30} alkenyl, C_{1-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or

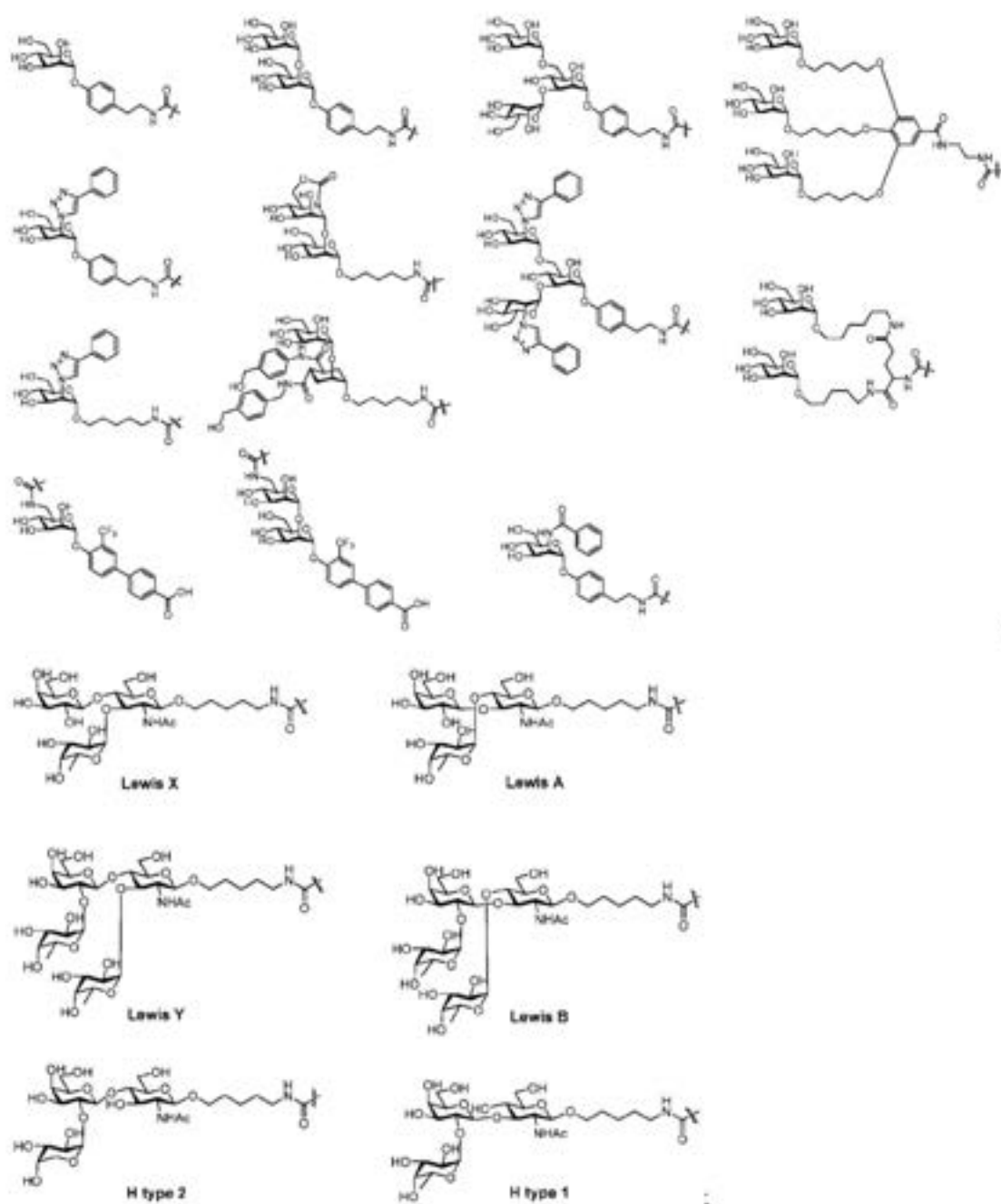
$-(CH_2)_nX_4$, n is 0 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when X_4 is a heterocyclic group, the heterocyclic group comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N, or a combination thereof; and

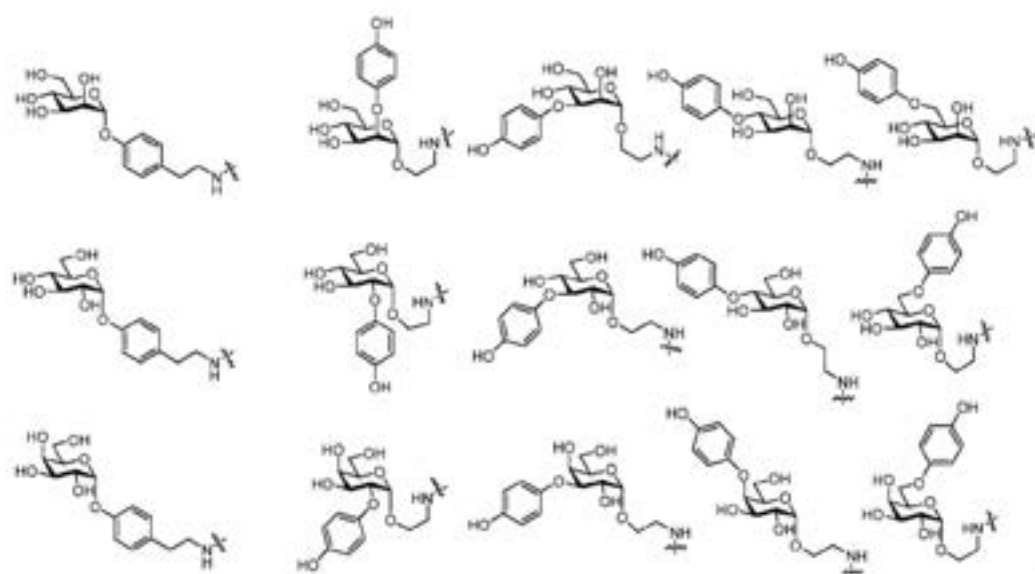
wherein X_3 is hydrogen, C_{1-6} alkyl, or hydroxyl.

2. The compound of claim 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.
3. The compound of claim 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.
4. The compound of claim 3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.
5. The copolymer of claim 3 or claim 4, wherein the PEG moiety is linear.
6. The compound of any one of claims 1 to 5, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.
7. The compound of any one of claims 1 to 6, wherein the glycosyl group comprises a terminal mannoside, a terminal fucoside, or both.

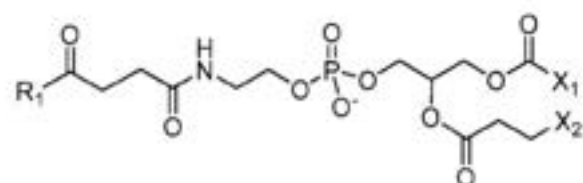
8. The compound of any one of claims 1 to 7, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
9. The compound of claim 8, wherein the tri-mannoside is a linear or branched tri-mannoside.
10. The compound of claim 9, wherein the branched tri-mannoside is a α -1,3- α -1,6-trimannoside.
11. The compound of any one of claims 1 to 10, wherein R_1 is a substituted glycosyl group.
12. The compound of claim 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.
13. The compound of claim 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.
14. The compound of claim 12 or claim 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof.
15. The compound of claim 12 or claim 13, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.
16. The compound of any one of claims 1 to 15, wherein R_1 is selected from the group consisting of:





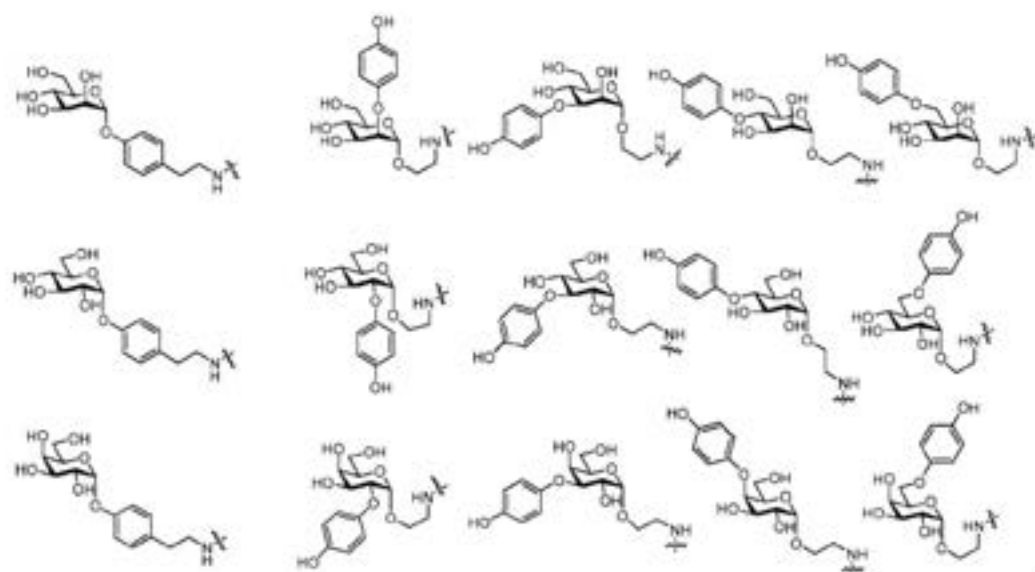


17. The compound of any one of claims 1 to 16, wherein the compound is of Formula 1.
18. The compound of any one of claims 1 to 16, wherein the compound is of Formula 2.
19. The compound of claim 18, wherein the compound is of Formula 3:

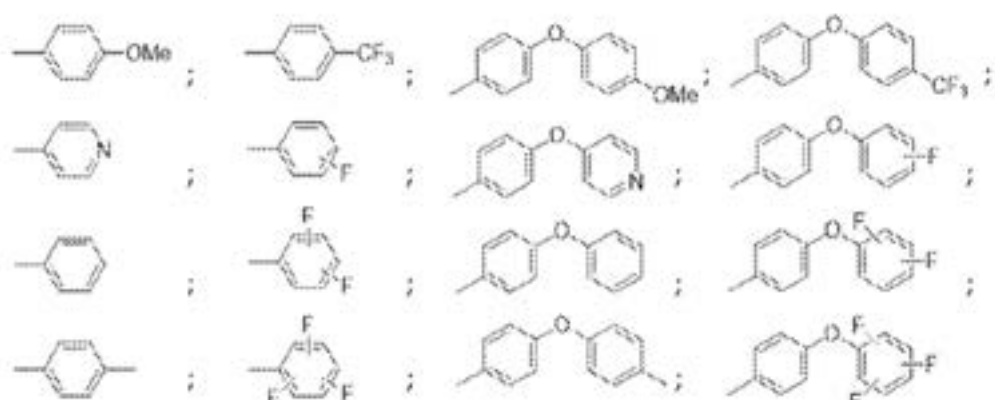


Formula 3; and

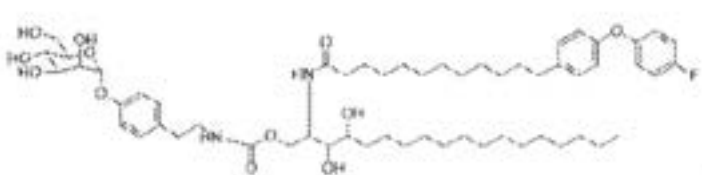
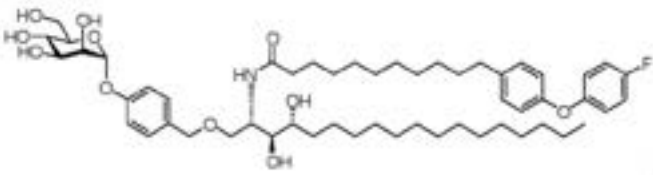
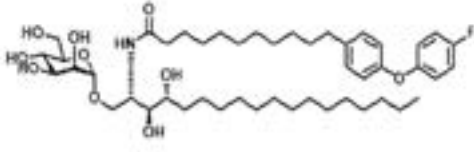
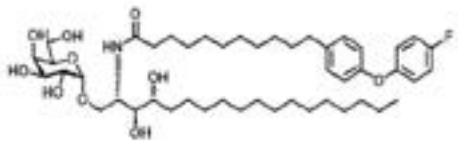
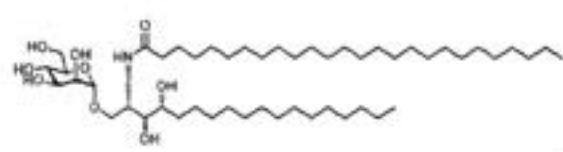
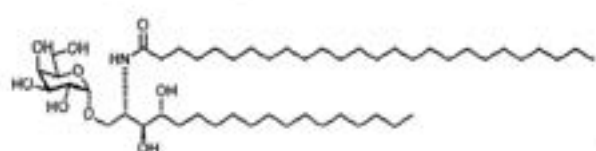
wherein R₁ is selected from the group consisting of:

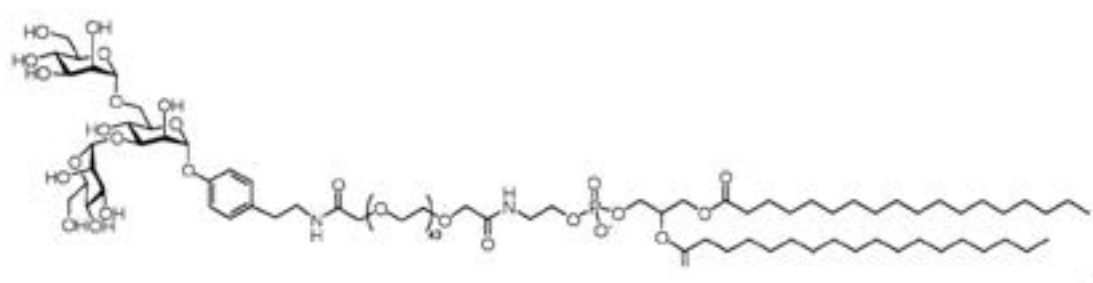
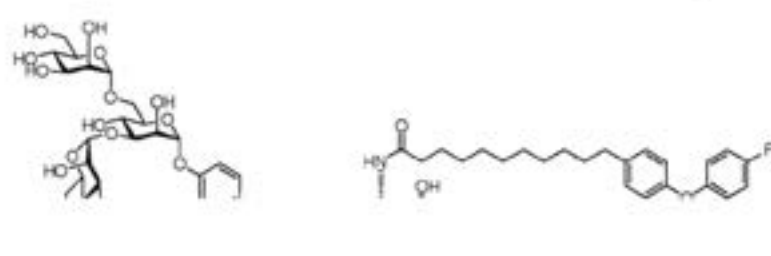
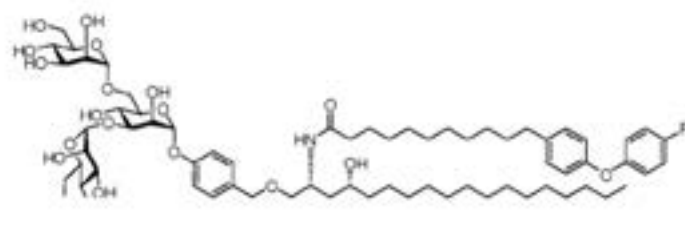
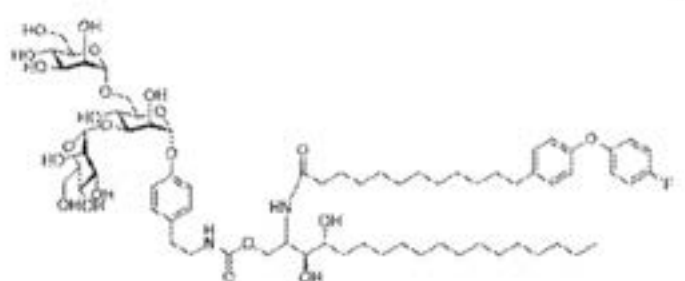
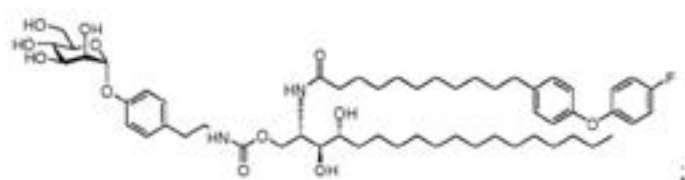


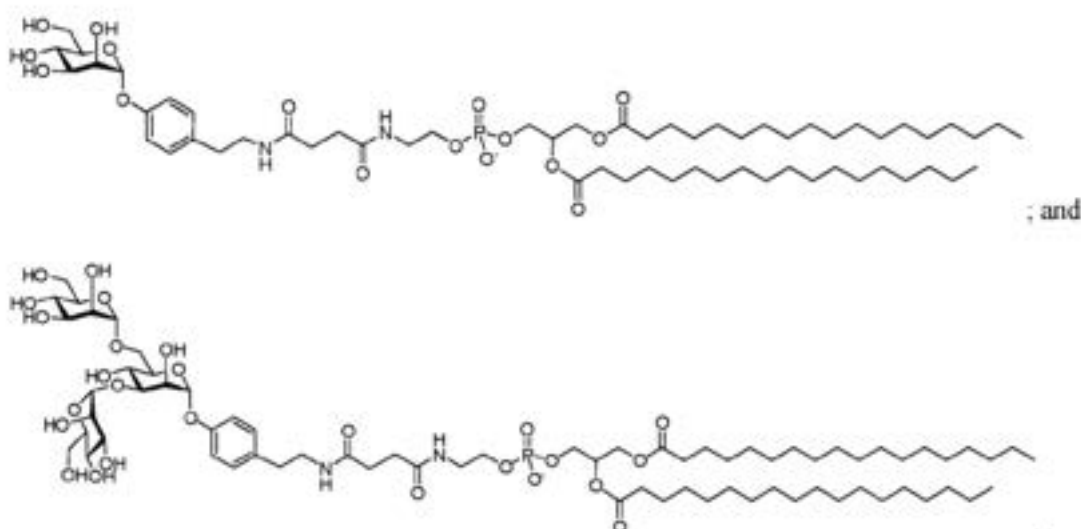
20. The compound of any one of claims 1 to 19, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons.
21. The compound of any one of claims 1 to 20, wherein X_1 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.
22. The compound of claim 21, wherein 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.
23. The compound of any one of claims 1 to 22, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen.
24. The compound of any one of claims 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.
25. The compound of claim 24, wherein the substituent is CH_3 , CF_3 , F, or OCH_3 .
26. The compound of claim 24 or claim 25, wherein X_4 comprises 1 to 3 substituents.
27. The compound of any one of claims 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.
28. The compound of any one of claims 1 to 27, wherein one of X_1 and X_2 is C_{15-30} alkyl, and the other one is $-(CH_2)_nX_4$.
29. The compound of any one of claims 1 to 28, wherein X_4 is selected from the group consisting of:



30. The compound of any one of claims 1 to 29, selected from the group consisting of:







31. The component of any one of claims 1 to 30, wherein the component is not glycolipid C34 or α -galactosylceramide.
32. A formulation for forming a lipid nanoparticle, comprising the compound of any one of claims 1 to 31, wherein the compound comprises 1 to 10 mol% of the composition.
33. The formulation of claim 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.
34. The formulation of claim 33, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
35. The formulation of claim 33 or claim 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.
36. The formulation of claim 35, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.
37. The formulation of claim 35 or claim 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, (3 β ,5 β)-3-hydroxycholestan-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.

38. The formulation of any one of claims 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.
39. The formulation of any one of claims 33 to 38, further comprising a payload.
40. The formulation of claim 39, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
41. The formulation of claim 40, wherein the nucleic acid is RNA or DNA.
42. The formulation of claim 41, wherein the payload encodes a polypeptide.
43. The formulation of any one of claims 39 to 42, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
44. The formulation of any one of claims 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.
45. The formulation of claim 44 or claim 45, wherein the first payload and the second payload are different.
46. The formulation of any one of claims 32 to 45, further comprising a pharmaceutically acceptable excipient, adjuvant, or a combination thereof.
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 claims 1 to 31.
48. The lipid nanoparticle of claim 47, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof.
49. The lipid nanoparticle of claim 47 or claim 48, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.
50. The lipid nanoparticle of claim 49, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
51. The lipid nanoparticle of claim 49 or claim 50, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.
52. The lipid nanoparticle of claim 51, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

53. The lipid nanoparticle of claim 51 or claim 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, (3 β ,5 β)-3-hydroxycholelan-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.
54. The lipid nanoparticle of any one of claims 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.
55. The lipid nanoparticle of any one of claims 47 to 54, wherein the membrane encapsulates a payload.
56. The lipid nanoparticle of claim 55, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
57. The lipid nanoparticle of claim 56, wherein the nucleic acid is RNA or DNA.
58. The lipid nanoparticle of claim 57, wherein the payload encodes a polypeptide.
59. The lipid nanoparticle of any one of claims 55 to 58, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
60. The lipid nanoparticle of any one of claims 55 to 59, wherein the payload is a first payload, and the membrane further encapsulates a second payload.
61. The lipid nanoparticle of claim 60, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
62. The lipid nanoparticle of claim 60 or claim 61, wherein the first payload and the second payload are different.
63. The lipid nanoparticle of any one of claims 47 to 62, being made from the composition of any one of claims 29 to 43.
64. The lipid nanoparticle of any one of claims 47 to 63, wherein the membrane is a bi-layer structure.
65. The lipid nanoparticle of any one of claims 47 to 64, having a diameter of 0.01 to 5 microns.
66. The lipid nanoparticle of any one of claims 47 to 65, wherein the plurality of the lipid components does not comprise glycolipid C34 or α -galactosylceramide (α -GalCer).
67. A formulation, comprising the lipid nanoparticle of any one of claims 47 to 66.
68. The formulation of claim 67, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.
69. The formulation of claim 67 or claim 68, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.

70. The formulation of claim 69, wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.
71. The formulation of any one of claims 67 to 70, further comprising an excipient, an adjuvant, or a combination thereof.
72. The formulation of claim 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.
73. The formulation of claim 71 or claim 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.
74. A kit for preparing a lipid nanoparticle, comprising:
a first reagent, comprising the compound of any one of claims 1 to 31; and
a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.
75. The kit of claim 74, wherein the second reagent comprises the ionizable lipid.
76. The kit of claim 75, wherein the kit further comprises a third reagent comprising a helper lipid.
77. The kit of any one of claims 74 to 76, wherein ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxylhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
78. The kit of any one of claims 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.
79. The kit of claim 78, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.
80. The kit of claim 78 or claim 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, (3 β ,5 β)-3-hydroxycholesterol-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.
81. The kit of any one of claims 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.

82. The kit of any one of claims 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.
83. The kit of claim 82, wherein the nucleic acid is RNA or DNA.
84. The kit of claim 83, wherein the payload encodes a polypeptide.
85. The kit of any one of claims 82 to 84, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
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 claims 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.
87. The method of claim 86, wherein the nucleic acid is RNA or DNA.
88. The method of claim 87, wherein the payload encodes a polypeptide.
89. The method of any one of claims 86 to 88, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
90. The method of any one of claims 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.
91. The method of claim 90, wherein the first payload and the second payload are different.
92. The method of any one of claims 86 to 91, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
93. The method of claim 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.
94. The method of claim 92 or claim 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.
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 claims 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.
96. The method of claim 95, wherein the nucleic acid is RNA or DNA.

97. The method of claim 96, wherein the payload encodes a polypeptide.
98. The method of any one of claims 95 to 97, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
99. The method of any one of claims 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.
100. The method of claim 99, wherein the first payload and the second payload are different.
101. The method of any one of claims 95 to 100, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
102. The method of claim 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.
103. The method of claim 101 or claim 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.
104. The method of any one of claims 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.
105. The method of claim 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.
106. The method of any one of claims 95 to 105, wherein the effective amount ranges from about 5 μ g to 1000 μ g.
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 claims 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.
108. The method of claim 107, wherein the nucleic acid is RNA or DNA.
109. The method of claim 108, wherein the payload encodes a polypeptide.
110. The method of any one of claims 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.
111. The method of claim 110, wherein the first payload and the second payload are different.

112. The method of any one of claims 107 to 111, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
113. The method of claim 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.
114. The method of claim 112 or claim 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.
115. The method of any one of claims 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.
116. The method of claim 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.
117. The method of any one of claims 107 to 116, wherein the effective amount ranges from about 5 μ g to 1000 μ g.

ABSTRACT

The present disclosure relates to novel compounds, methods, and cell targeting formulations, e.g., a lipid nanoparticle (LNP) for targeted delivery to a tissue or a cell type. The compound and formulation provided herein are designed to have a targeting moiety configured to provide selective delivery features for the formulation and a lipid tail for being incorporated into the bilayer membrane of the formed lipid nanoparticle.



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
63/575,093

RECEIPT DATE / TIME
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A1000-01100P32

Title of Invention
METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

Application Information

APPLICATION TYPE
Utility - Provisional Application under
35 USC 111(b)

PATENT #
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CONFIRMATION #
2760

FILED BY
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PATENT CENTER #
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FILING DATE
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CUSTOMER #
169053

FIRST NAMED INVENTOR
Chi-Huey WONG

CORRESPONDENCE ADDRESS
-

AUTHORIZED BY
Wade Lin

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
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National Stage of an International Application under 35 U.S.C. 371

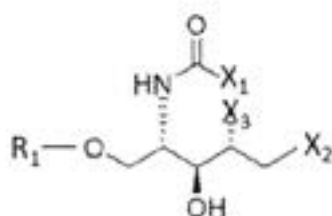
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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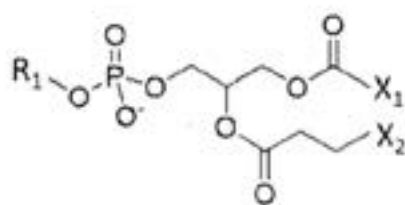
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1910), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

What is claimed is:

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;

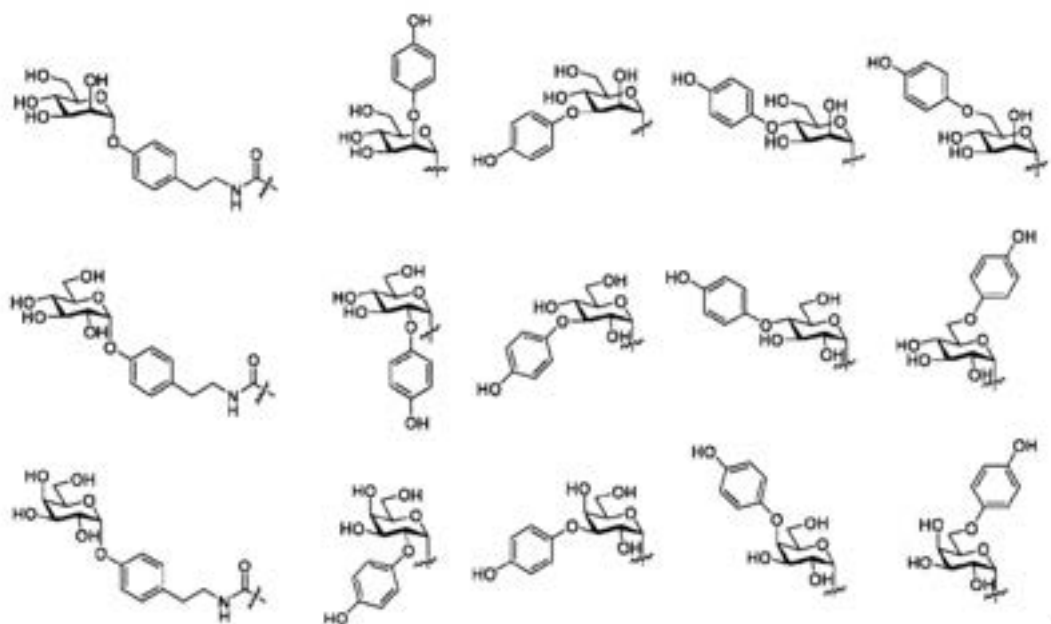
wherein X_1 and X_2 are each independently hydrogen, C_{1-30} alkyl, C_{1-30} alkenyl, C_{1-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or

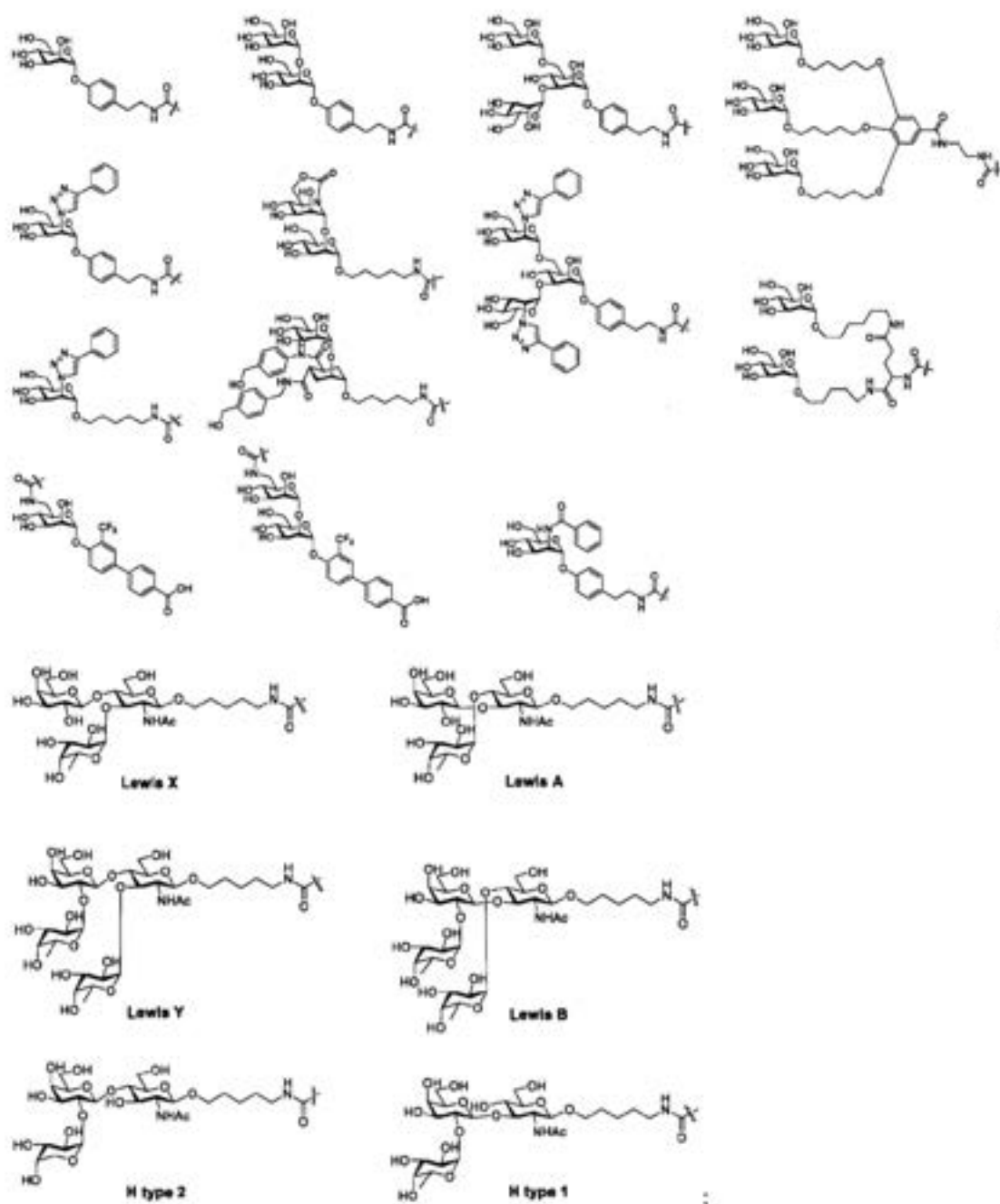
$-(CH_2)_nX_4$, n is 0 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when X_4 is a heterocyclic group, the heterocyclic group comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N, or a combination thereof; and

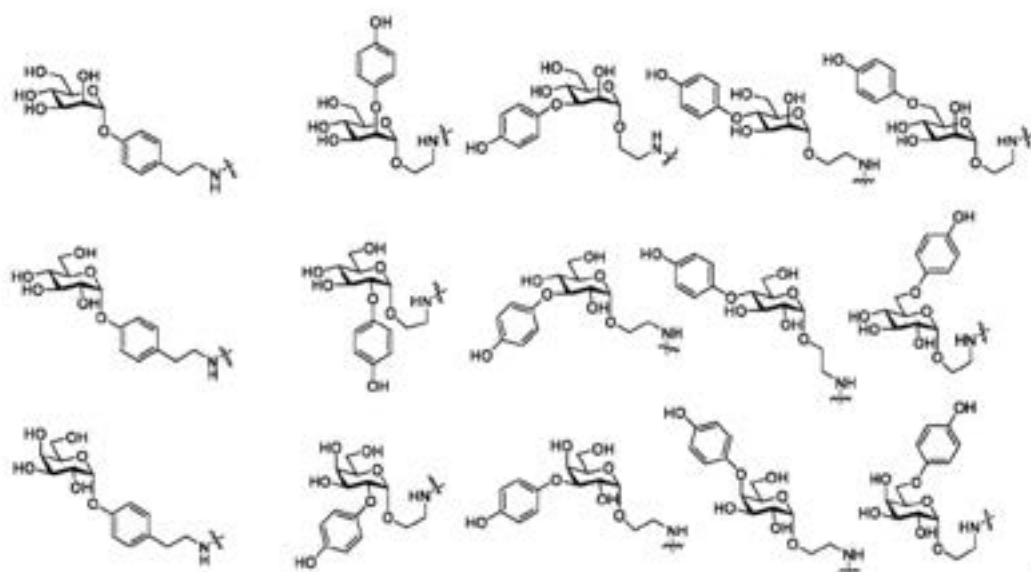
wherein X_3 is hydrogen, C_{1-6} alkyl, or hydroxyl.

2. The compound of claim 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.
3. The compound of claim 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.
4. The compound of claim 3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.
5. The copolymer of claim 3 or claim 4, wherein the PEG moiety is linear.
6. The compound of any one of claims 1 to 5, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.
7. The compound of any one of claims 1 to 6, wherein the glycosyl group comprises a terminal mannoside, a terminal fucoside, or both.

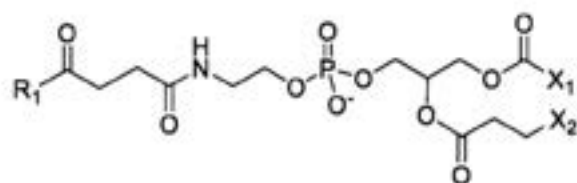
8. The compound of any one of claims 1 to 7, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
9. The compound of claim 8, wherein the tri-mannoside is a linear or branched tri-mannoside.
10. The compound of claim 9, wherein the branched tri-mannoside is a α -1,3- α -1,6-trimannoside.
11. The compound of any one of claims 1 to 10, wherein R_1 is a substituted glycosyl group.
12. The compound of claim 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.
13. The compound of claim 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.
14. The compound of claim 12 or claim 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.
15. The compound of claim 12 or claim 13, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.
16. The compound of any one of claims 1 to 15, wherein R_1 is selected from the group consisting of:





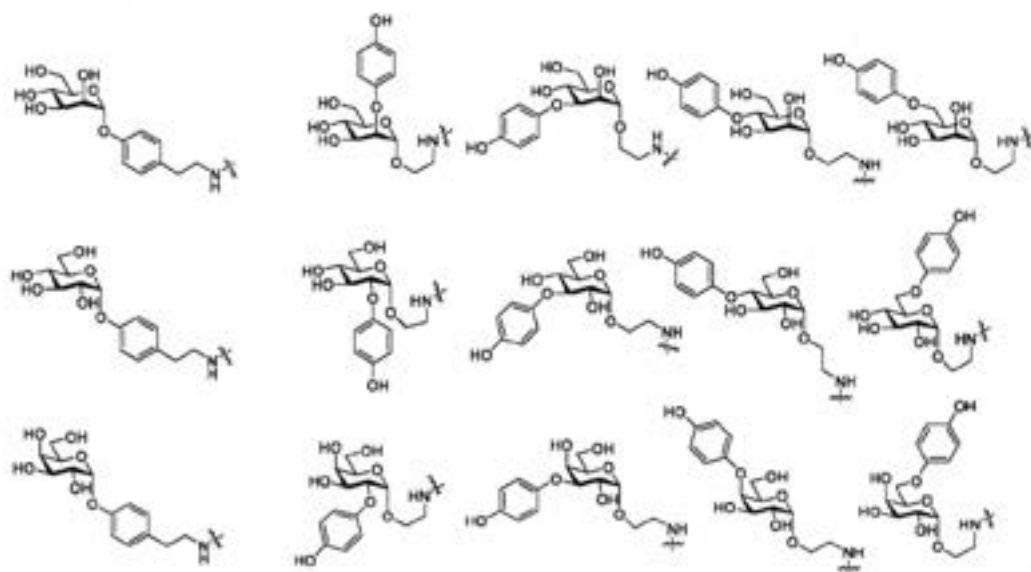


17. The compound of any one of claims 1 to 16, wherein the compound is of Formula 1.
18. The compound of any one of claims 1 to 16, wherein the compound is of Formula 2.
19. The compound of claim 18, wherein the compound is of Formula 3:

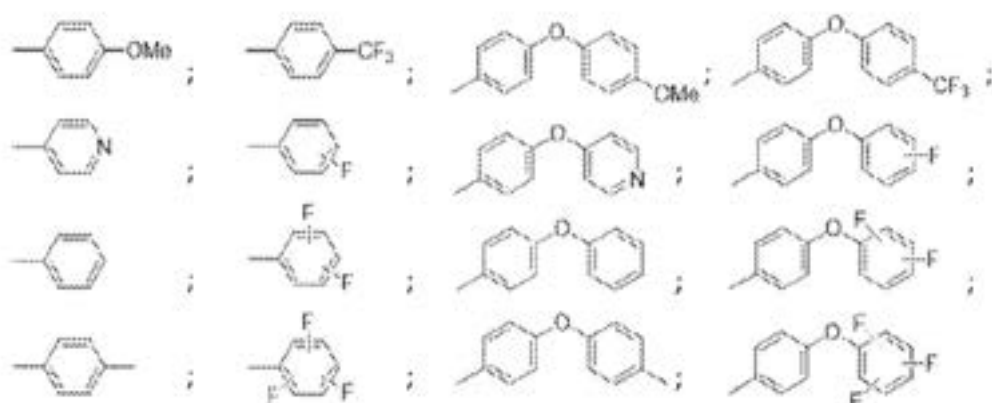


Formula 3; and

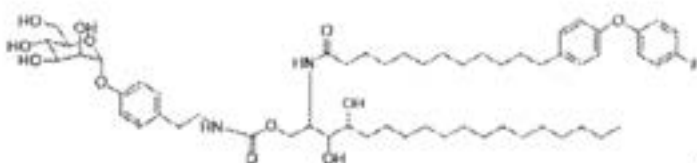
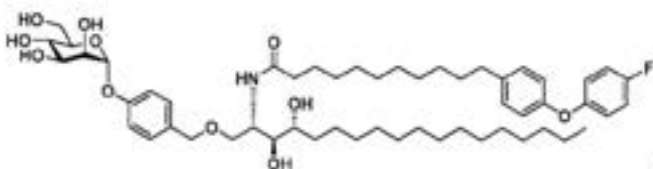
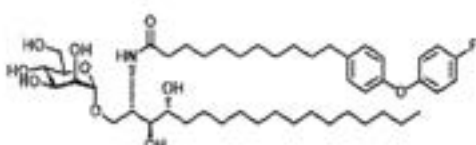
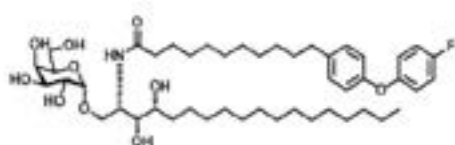
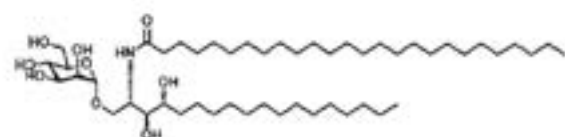
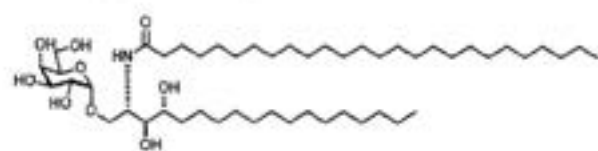
wherein R_1 is selected from the group consisting of:

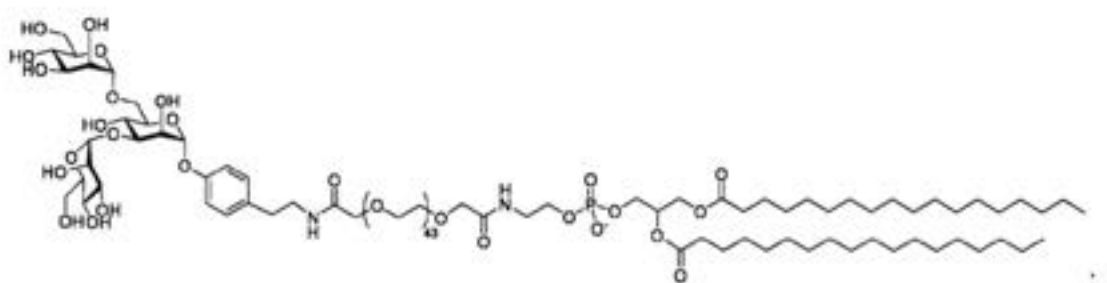
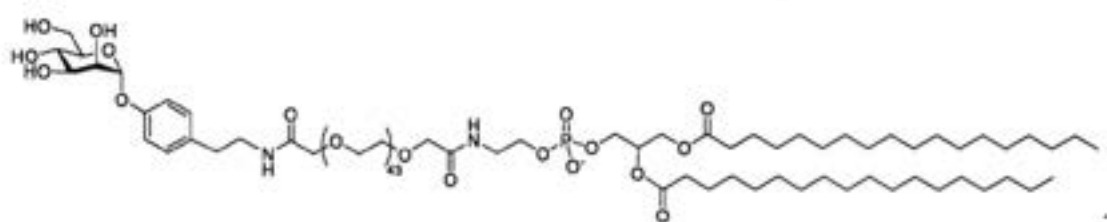
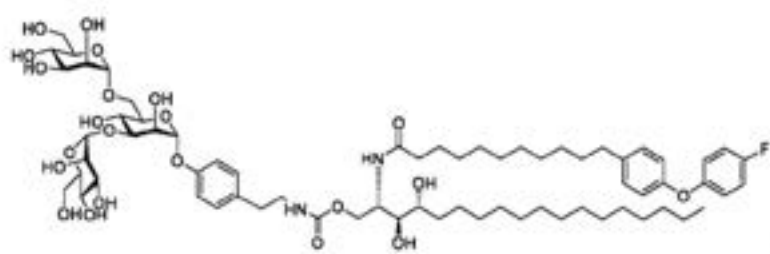
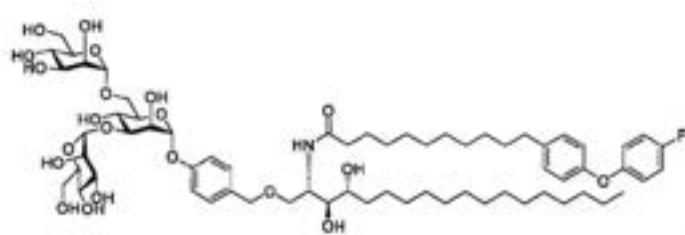
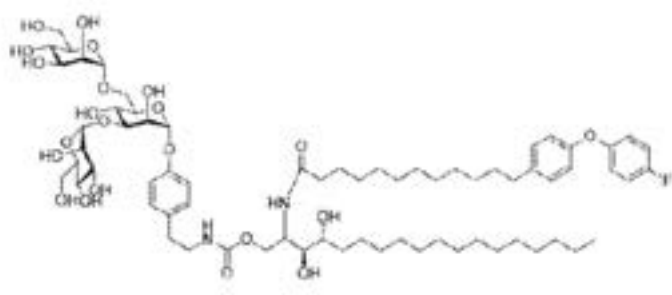
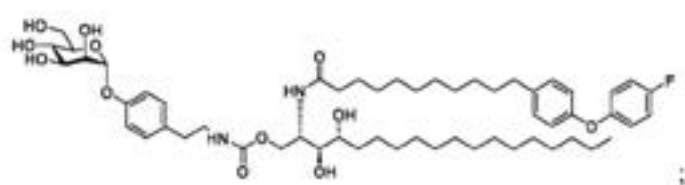


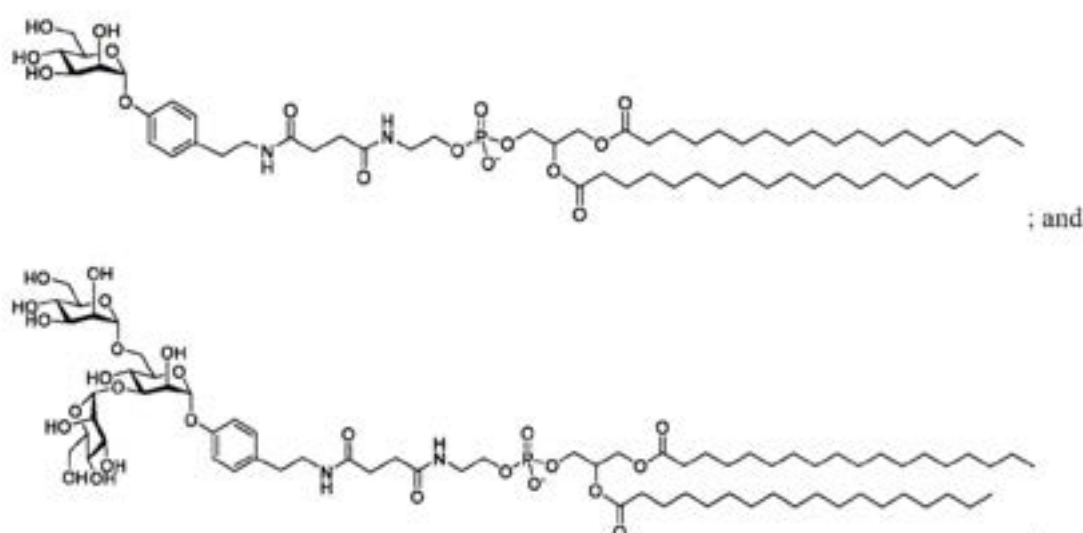
20. The compound of any one of claims 1 to 19, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons.
21. The compound of any one of claims 1 to 20, wherein X_1 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.
22. The compound of claim 21, wherein 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.
23. The compound of any one of claims 1 to 22, provided that when one of X_1 and X_2 is hydrogen, the other one is not hydrogen.
24. The compound of any one of claims 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.
25. The compound of claim 24, wherein the substituent is CH_3 , CF_3 , F, or OCH_3 .
26. The compound of claim 24 or claim 25, wherein X_4 comprises 1 to 3 substituents.
27. The compound of any one of claims 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.
28. The compound of any one of claims 1 to 27, wherein one of X_1 and X_2 is C_{15-30} alkyl, and the other one is $-(CH_2)_nX_4$.
29. The compound of any one of claims 1 to 28, wherein X_4 is selected from the group consisting of:



30. The compound of any one of claims 1 to 29, selected from the group consisting of:







31. The component of any one of claims 1 to 30, wherein the component is not glycolipid C34 or α -galactosylceramide.
32. A formulation for forming a lipid nanoparticle, comprising the compound of any one of claims 1 to 31, wherein the compound comprises 1 to 10 mol% of the composition.
33. The formulation of claim 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.
34. The formulation of claim 33, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
35. The formulation of claim 33 or claim 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.
36. The formulation of claim 35, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.
37. The formulation of claim 35 or claim 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, (3 β ,5 β)-3-hydroxycholestan-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.

38. The formulation of any one of claims 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.
39. The formulation of any one of claims 33 to 38, further comprising a payload.
40. The formulation of claim 39, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
41. The formulation of claim 40, wherein the nucleic acid is RNA or DNA.
42. The formulation of claim 41, wherein the payload encodes a polypeptide.
43. The formulation of any one of claims 39 to 42, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
44. The formulation of any one of claims 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.
45. The formulation of claim 44 or claim 45, wherein the first payload and the second payload are different.
46. The formulation of any one of claims 32 to 45, further comprising a pharmaceutically acceptable excipient, adjuvant, or a combination thereof.
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 claims 1 to 31.
48. The lipid nanoparticle of claim 47, wherein the plurality of the lipid components further comprises an ionizable lipid, a helper lipid, or a combination thereof.
49. The lipid nanoparticle of claim 47 or claim 48, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.
50. The lipid nanoparticle of claim 49, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecoxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
51. The lipid nanoparticle of claim 49 or claim 50, wherein the helper lipid comprises a phosphatidylcholine, a cholesterol or a derivative thereof, a polyethylene glycol-lipid (PEG-lipid), or a mixture thereof.
52. The lipid nanoparticle of claim 51, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.

53. The lipid nanoparticle of claim 51 or claim 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, (3 β ,5 β)-3-hydroxycholestan-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.
54. The lipid nanoparticle of any one of claims 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.
55. The lipid nanoparticle of any one of claims 47 to 54, wherein the membrane encapsulates a payload.
56. The lipid nanoparticle of claim 55, wherein the payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
57. The lipid nanoparticle of claim 56, wherein the nucleic acid is RNA or DNA.
58. The lipid nanoparticle of claim 57, wherein the payload encodes a polypeptide.
59. The lipid nanoparticle of any one of claims 55 to 58, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
60. The lipid nanoparticle of any one of claims 55 to 59, wherein the payload is a first payload, and the membrane further encapsulates a second payload.
61. The lipid nanoparticle of claim 60, wherein the second payload is a nucleic acid, a compound, a polypeptide, a protein, a glycan, or a combination thereof.
62. The lipid nanoparticle of claim 60 or claim 61, wherein the first payload and the second payload are different.
63. The lipid nanoparticle of any one of claims 47 to 62, being made from the composition of any one of claims 29 to 43.
64. The lipid nanoparticle of any one of claims 47 to 63, wherein the membrane is a bi-layer structure.
65. The lipid nanoparticle of any one of claims 47 to 64, having a diameter of 0.01 to 5 microns.
66. The lipid nanoparticle of any one of claims 47 to 65, wherein the plurality of the lipid components does not comprise glycolipid C34 or α -galactosylceramide (α -GalCer).
67. A formulation, comprising the lipid nanoparticle of any one of claims 47 to 66.
68. The formulation of claim 67, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.
69. The formulation of claim 67 or claim 68, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.

70. The formulation of claim 69, wherein the first lipid nanoparticle and the second lipid nanoparticle are different in size, membrane components, payload encapsulated therewithin, or a combination thereof.
71. The formulation of any one of claims 67 to 70, further comprising an excipient, an adjuvant, or a combination thereof.
72. The formulation of claim 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.
73. The formulation of claim 71 or claim 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.
74. A kit for preparing a lipid nanoparticle, comprising:
a first reagent, comprising the compound of any one of claims 1 to 31; and
a second reagent, comprising an ionizable lipid, a helper lipid, or a mixture thereof.
75. The kit of claim 74, wherein the second reagent comprises the ionizable lipid.
76. The kit of claim 75, wherein the kit further comprises a third reagent comprising a helper lipid.
77. The kit of any one of claims 74 to 76, wherein ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
78. The kit of any one of claims 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.
79. The kit of claim 78, wherein the phosphatidylcholine comprises distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylethanolamine (DPOE), or a mixture thereof.
80. The kit of claim 78 or claim 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, (3 β ,5 β)-3-hydroxycholestan-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.
81. The kit of any one of claims 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.

82. The kit of any one of claims 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.
83. The kit of claim 82, wherein the nucleic acid is RNA or DNA.
84. The kit of claim 83, wherein the payload encodes a polypeptide.
85. The kit of any one of claims 82 to 84, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
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 claims 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.
87. The method of claim 86, wherein the nucleic acid is RNA or DNA.
88. The method of claim 87, wherein the payload encodes a polypeptide.
89. The method of any one of claims 86 to 88, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
90. The method of any one of claims 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.
91. The method of claim 90, wherein the first payload and the second payload are different.
92. The method of any one of claims 86 to 91, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
93. The method of claim 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.
94. The method of claim 92 or claim 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.
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 claims 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.
96. The method of claim 95, wherein the nucleic acid is RNA or DNA.

97. The method of claim 96, wherein the payload encodes a polypeptide.
98. The method of any one of claims 95 to 97, wherein the payload is immunogenic, or the payload is a nucleic acid configured to encode an immunogenic polypeptide or protein.
99. The method of any one of claims 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.
100. The method of claim 99, wherein the first payload and the second payload are different.
101. The method of any one of claims 95 to 100, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
102. The method of claim 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.
103. The method of claim 101 or claim 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.
104. The method of any one of claims 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.
105. The method of claim 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.
106. The method of any one of claims 95 to 105, wherein the effective amount ranges from about 5 μ g to 1000 μ g.
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 claims 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.
108. The method of claim 107, wherein the nucleic acid is RNA or DNA.
109. The method of claim 108, wherein the payload encodes a polypeptide.
110. The method of any one of claims 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.
111. The method of claim 110, wherein the first payload and the second payload are different.

112. The method of any one of claims 107 to 111, wherein the lipid nanoparticle is administered together with an excipient, an adjuvant, or a combination thereof.
113. The method of claim 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.
114. The method of claim 112 or claim 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.
115. The method of any one of claims 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.
116. The method of claim 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.
117. The method of any one of claims 107 to 116, wherein the effective amount ranges from about 5 μ g to 1000 μ g.

ABSTRACT

The present disclosure relates to novel compounds, methods, and cell targeting formulations, e.g., a lipid nanoparticle (LNP) for targeted delivery to a tissue or a cell type. The compound and formulation provided herein are designed to have a targeting moiety configured to provide selective delivery features for the formulation and a lipid tail for being incorporated into the bilayer membrane of the formed lipid nanoparticle.

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METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

FIELD

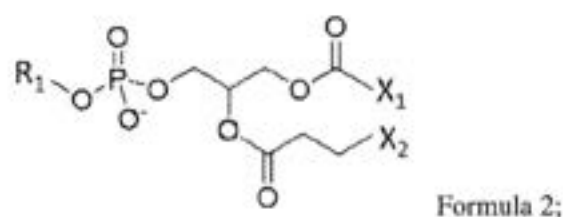
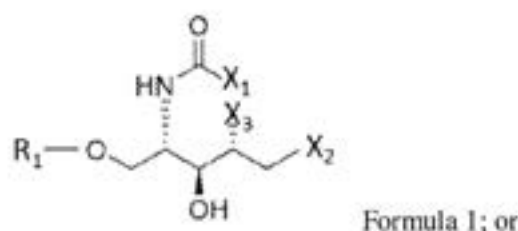
[0001] The instant disclosure relates to novel formulations for targeted nano-delivery. Specifically, the disclosure relates to compositions and methods directed to novel bi-functional nanoparticle formulations capable of selectively delivering a payload to a desired region in a tissue or a specific cell type, including dendritic cells.

BACKGROUND OF THE INVENTION

[0001] 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

[0002] 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:



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.

[0003] 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.

[0004] 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.

[0005] 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.

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] 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

[0011] 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.

[0012] FIG. 2 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 uptake of the analysis demonstrating the efficacy 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.

[0013] 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. 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).

[0014] 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 according to embodiments of the present disclosure targeting BDMCs compared with LNPs without the compound of the present disclosure. The FITC+ values shown in the figures are fluorescent intensities in arbitrary units. 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).

[0015] 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 tested animals. The assay generates 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.

[0016] FIG. 6 shows the ^1H NMR spectrum of compound 12 of the present disclosure.

[0017] FIG. 7 shows the ^{13}C NMR spectrum of compound 12 of the present disclosure.

[0018] FIG. 8 presents bar charts showing the IFN γ (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.

[0019] 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 using different dilution factors to show the difference between samples.

[0020] 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.

[0021] 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.

[0022] FIG. 12 shows the LCMS spectrum of compound 21 of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

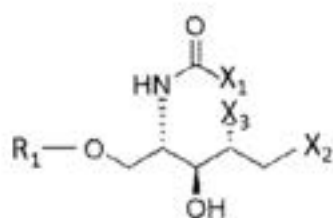
[0023] Nanoparticles have been widely used in various applications. Among them, **lipid nanoparticles (e.g., liposomes)** are the most well-established delivery systems for medicines due to their biocompatibility and biodegradability. A typical liposome has a bilayer structure formed by phospholipids 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.

[0024] 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.

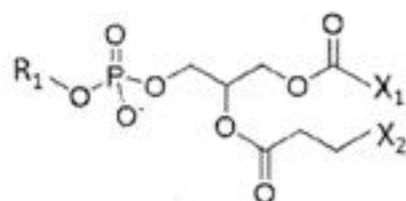
[0025] **TARGETING LIPID COMPOUND**

[0026] 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 structural moiety, which can be incorporated into a lipid bi-layer such as an LNP. 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 compound, which comprises two extended structures (e.g., X₁ and X₂ 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.

[0027] 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.

[0028] **R_1 group**

[0029] **Targeting Functionality.** In certain embodiments, the R_1 group is configured to provide selective 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 receptor on a target cell. For example, the R_1 group might be configured to target the DC-SIGN of a dendritic cell.

[0030] 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.

[0031] 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- α 2,3-Gal-GlcNAc. In some

embodiments, the R_1 group is configured to target Siglec-2, and the glycosyl group can comprise 9-Biphenyl Neu5Ac- α 2,6-Gal-GlcNAc. In some embodiments, the R_1 group is configured to target Siglec-5/E, and the glycosyl group can comprise Neu5Ac- α 2,3-Gal-GlcNAc.

[0032] In some embodiments, the R_1 group 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 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, wherein the substituent of the aryl is C_{1-6} alkyl, halide, or C_{1-6} alkyl halide. 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.

[0033] *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.

[0034] *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.

[0035] 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.

[0036] *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.

[0037] 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_{1-6} 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. 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).

[0038] 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.

[0039] 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof. For example, the substituted benzene ring can be a phenol group.

[0040] 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}

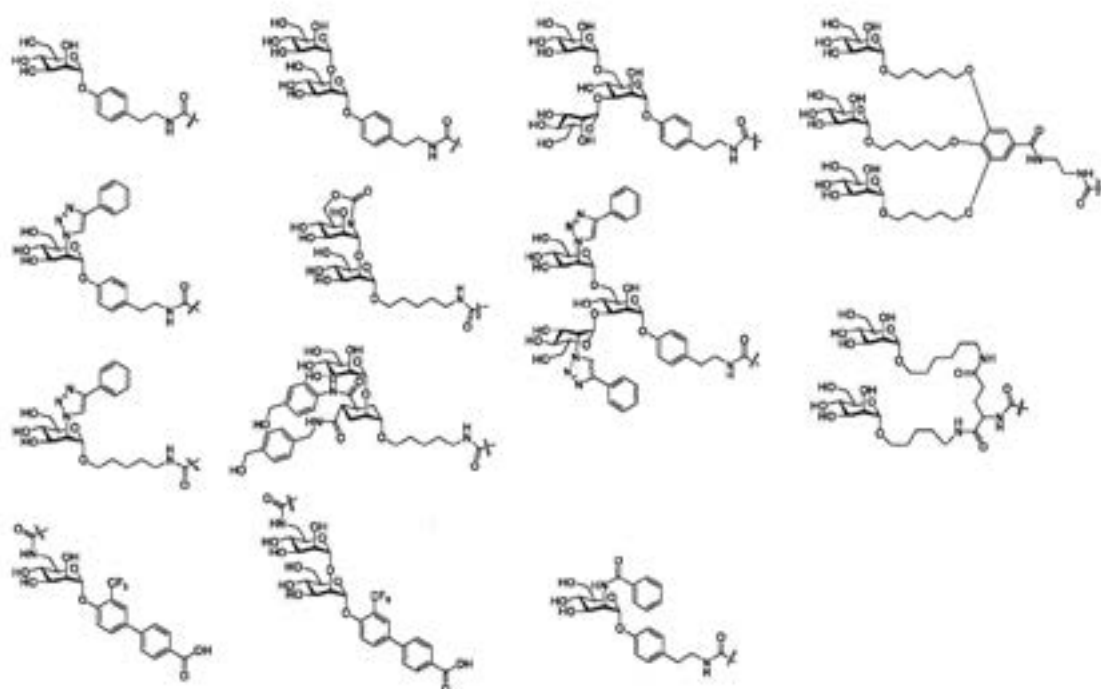
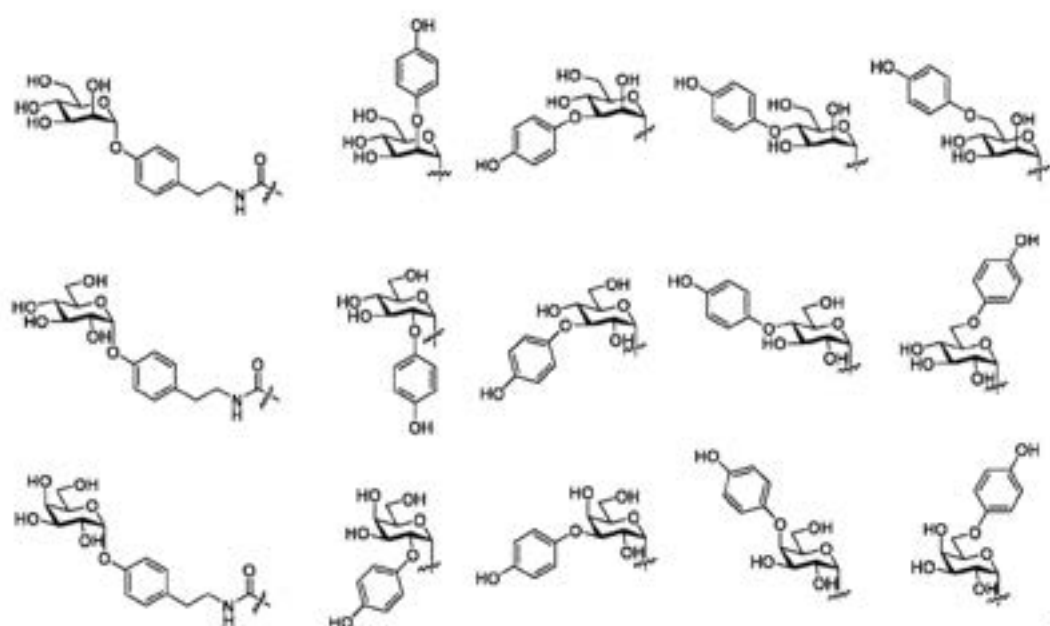
alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof. In certain embodiments, the R_1 group is a mono-mannoside substituted with a first substitute and a second substitute; each of the first substitute and the second substitute is 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.

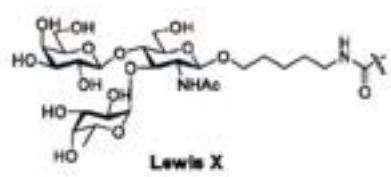
[0041] In some embodiments, the R_1 group comprises a first mannoside and a second mannoside.. Each of the first mannoside and the second mannoside is independently substituted with 1 to 6 substituents, and each substituent can be C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, amine, C_{1-6} alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

[0042] *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.

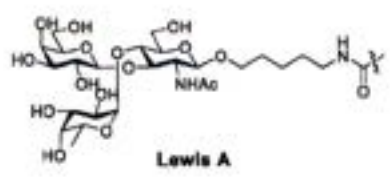
[0043] In some other embodiments, the K_D at pH 5 can be 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1250, 1500, 1750, or 2000 nM, or any range defined by the foregoing endpoints, such as, 1 to 2000, 1 to 1500, 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.

[0044] *Examples.* In some embodiments, the R_1 group is selected from the group consisting of:

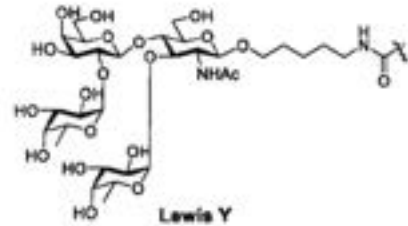




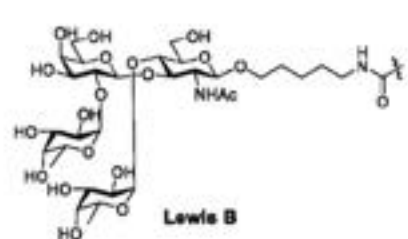
Lewis X



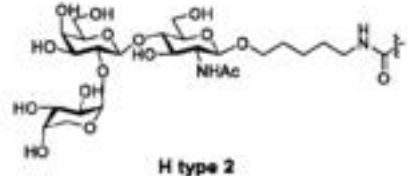
Lewis A



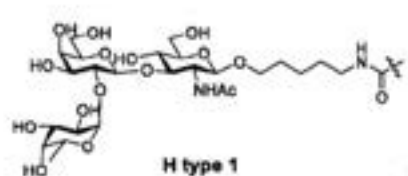
Lewis Y



Lewis B

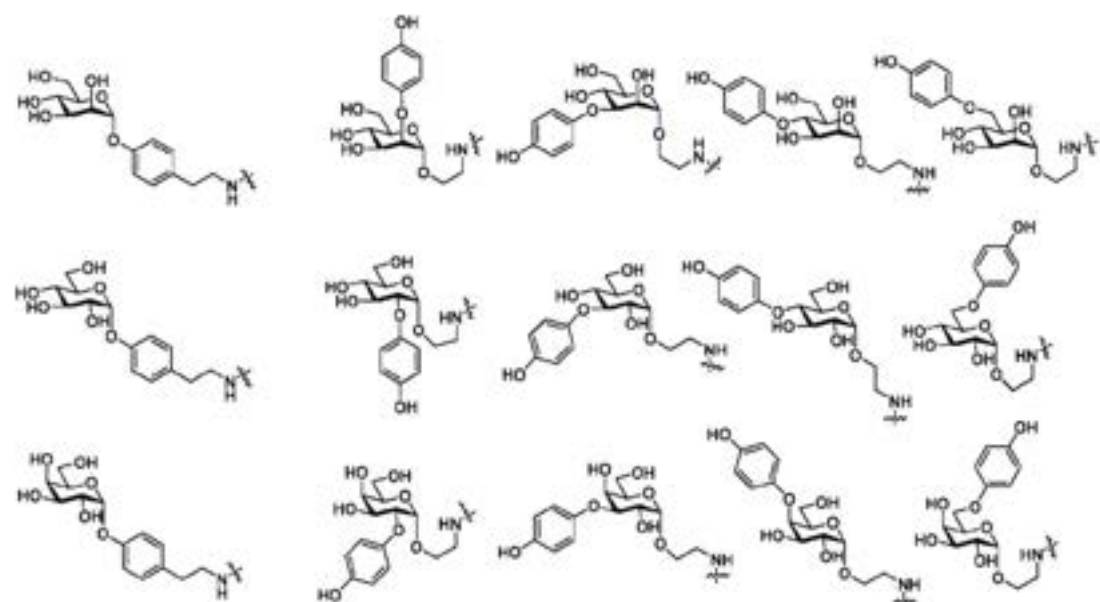


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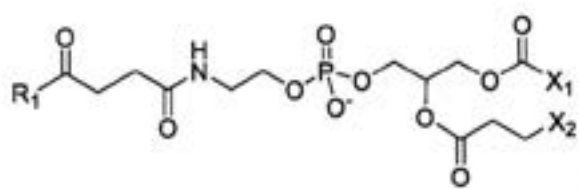


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; and

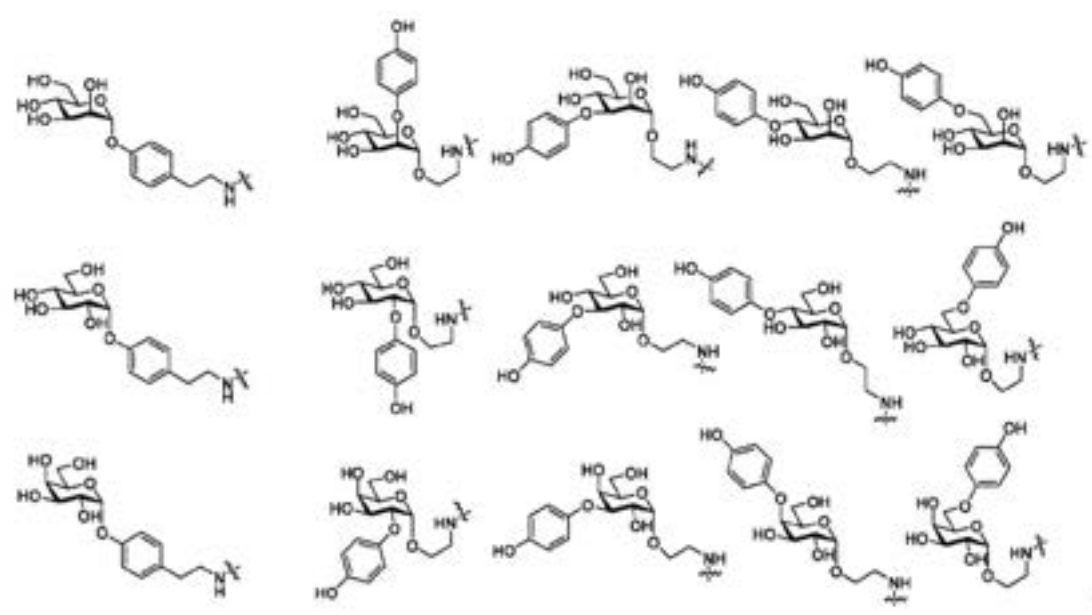


[0045] In some embodiments, the compound of the present disclosure has the structure shown in Formula 3:



Formula 3; and

wherein the R₁ group is selected from the group consisting of:



[0046] *X₁ and X₂*

[0047] The X₁ and X₂ are each independently hydrogen, C₁₋₃₀ alkyl, C₁₋₃₀ alkenyl, C₁₋₃₀ alkynyl, aryl, aryloxy, or a substituted version thereof, or -(CH₂)_nX₄, n is 0 to 30, and X₄ is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided that when X₄ 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 X₁ and X₂ groups is designed to provide the compound of the present disclosure with desired hydrophobicity.

[0048] In some embodiments, at least one of the X₁ and X₂ 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.

[0049] 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.

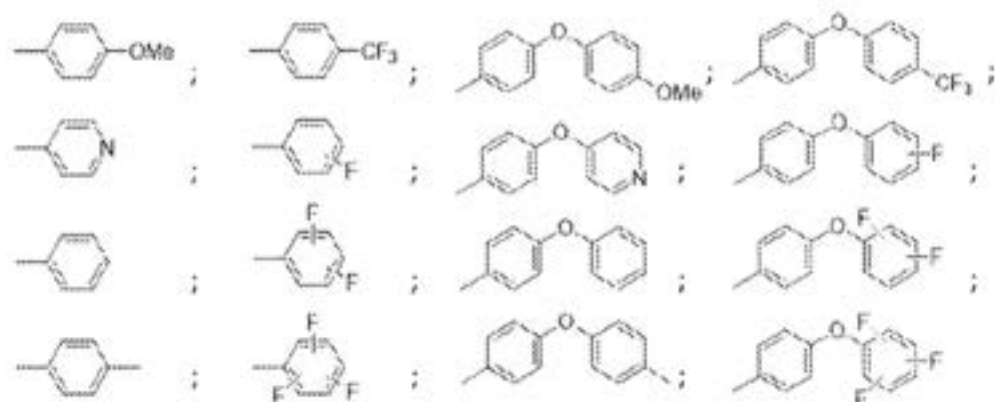
[0050] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{8-30} alkyl, C_{8-30} alkenyl, C_{8-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)_nX_4$, n is 8 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided 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.

[0051] 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.

[0052] 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 .

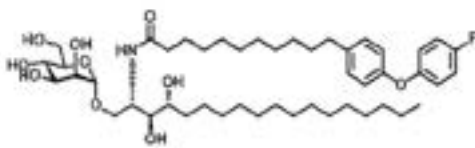
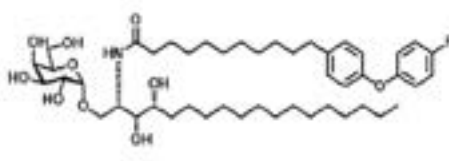
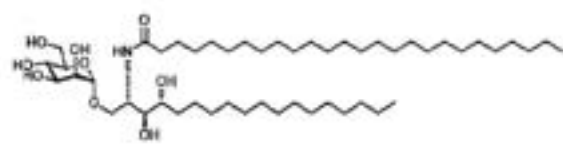
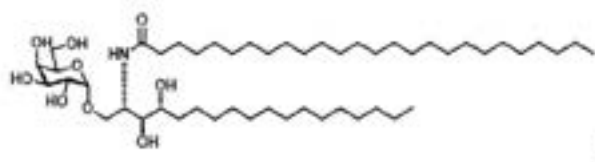
[0053] In some embodiments, X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

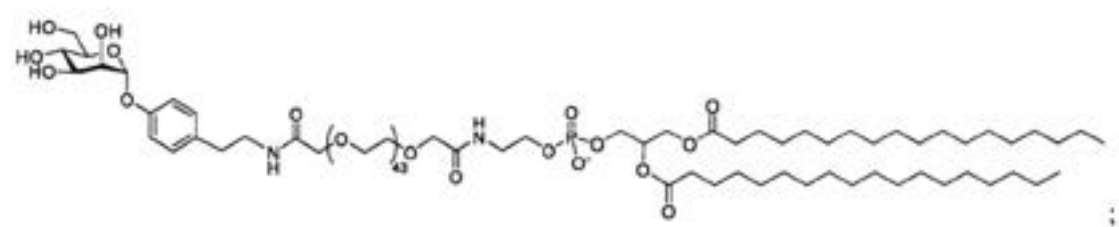
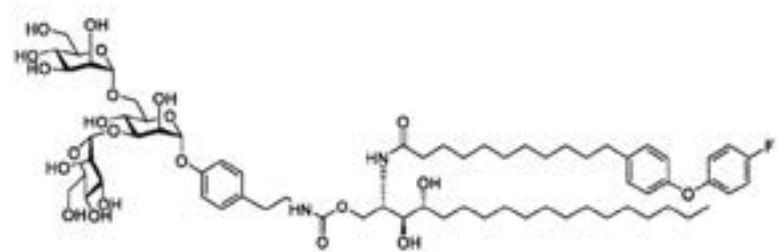
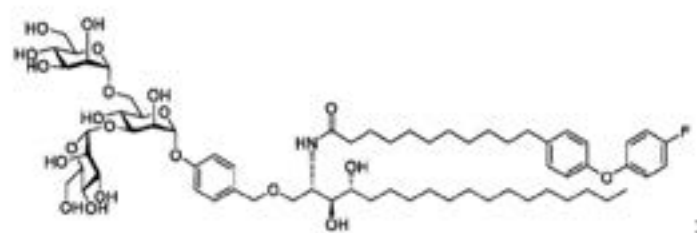
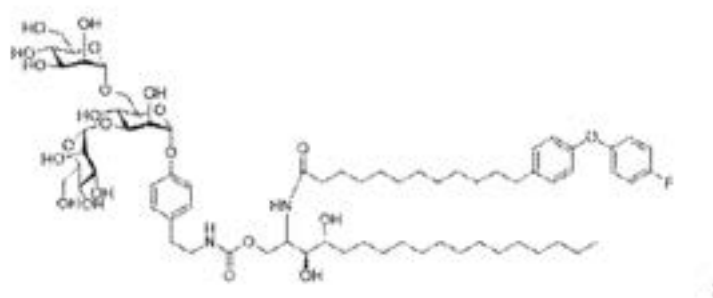
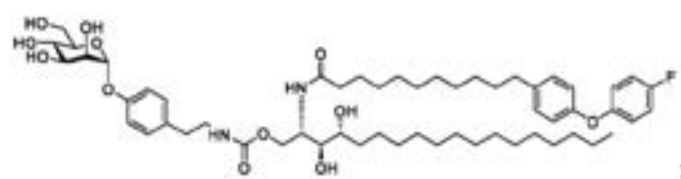
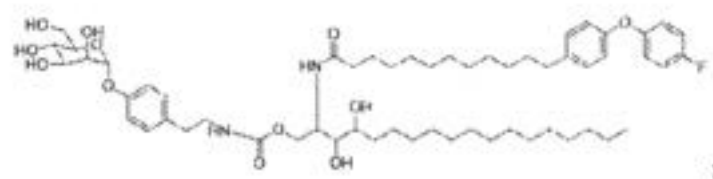
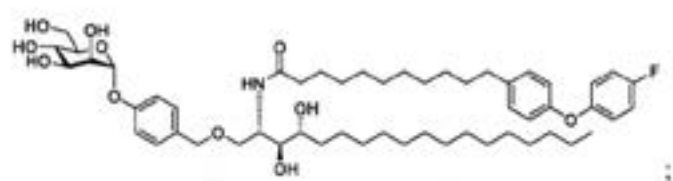
[0054] In certain embodiments, X_4 is selected from the group consisting of:

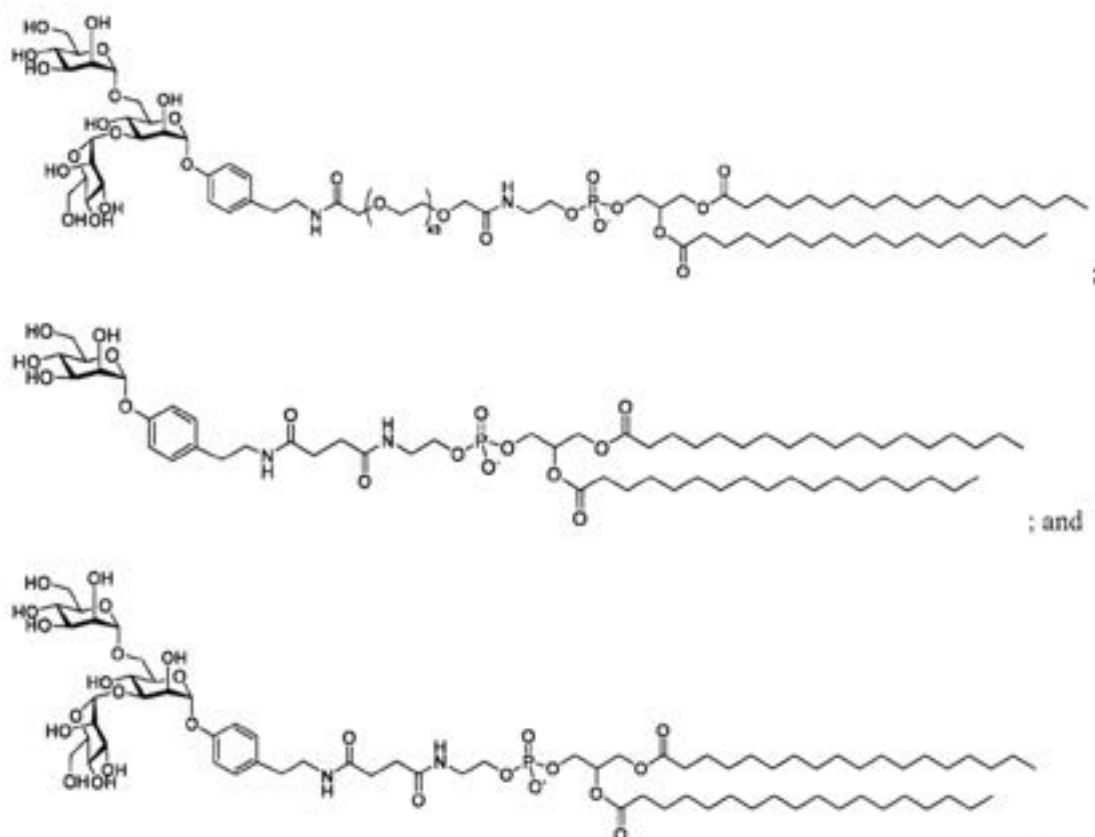


[0055] **Exemplary Compound of the Present Disclosure**

[0056] 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).







[0057] **COMPOSITION FOR FORMING A LIPID NANOPARTICLE**

[0058] 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.

[0059] 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 R₁

group, different targeting moiety of the R₁ group targeting the same target, or different targeting moiety of the R₁ group targeting different targets.

[0060] 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.

[0061] 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.

[0062] ***Ionizable lipid.***

[0063] 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-undecoxylhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.

[0064] ***Helper lipid.***

[0065] A helper lipid is a lipid used to increase particle stability and fluidity of lipid nanoparticles. A helper 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.

[0066] 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.

[0067] *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.

[0068] *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, dhydroergosterol, 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, (3 β ,5 β)-3-hydroxycholestan-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.

[0069] *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 R₁ comprises a PEG moiety. In those circumstances, the composition does not need to comprise a PEG-lipid.

[0070] *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.

[0071] 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%

[0072] **LIPID NANOPARTICLE (LNP)**

[0073] 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%.

[0074] 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.

[0075] 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%,.

[0076] *Size of the LNP.*

[0077] 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.

[0078] *Zeta potential and molecular weight.*

[0079] Without wishing to be bound by theories, the zeta potential and molecular weight of a polymersome may affect the cellular uptake of the polymersome. In some embodiments, the polymersome 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 polymersome 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.

[0080] *Payload*

[0081] 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.

[0082] 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.

[0083] In some embodiments, the polypeptide or the protein is immunogenic (e.g., antigenic) to an organism to which the polymersome is administered. In such embodiments, the polymersome 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., 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.

[0084] 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'-O 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.

[0085] 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).

[0086] 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.

[0087] 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.

[0088] 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.

[0089] In some embodiments, the N/P ratio (positively-chargeable polymer amine (N = nitrogen) groups to negatively-charged nucleic acid phosphate (P) groups) of the polymersome 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, a polymersome encapsulating a mRNA has a nanoparticle/mRNA (N/P) ratio of about 10 or about 20.

[0090] 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*.

[0091] ***Kit for Preparing a Lipid Nanoparticle.***

[0092] 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.

[0093] ***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.

[0094] **COMPOSITIONS/FORMULATIONS**

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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).

[0100] 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.

[0101] *Combination compositions*

[0102] 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.

[0103] 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.

[0104] 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.

[0105] *Additional components of the composition*

[0106] 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.

[0107] 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.).

[0108] 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.

[0109] 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).

[0110] 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).

[0111] 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.

[0112] **METHODS OF USE**

[0113] 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.

[0114] *Methods of targeted payload delivery in a subject*

[0115] 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.

[0116] *Methods of preventing or treating a disease in a subject*

[0117] 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.

[0118] 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 R₁ 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 R₁ 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.

[0119] 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.

[0120] 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.

[0121] *Methods of boosting an adaptive immune response*

[0122] 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.

[0123] 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 R₁ group of the compound comprises a structure that binds to an antigen-presenting cell with the desired specificity or affinity. For example, the R₁ 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.

[0124] 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.

[0125] *Administration*

[0126] 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.

[0127] *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.

[0128] *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.

[0129] 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).

[0130] 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.

[0131] 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.

[0132] 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%.

[0133] 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.

[0134] 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.

[0135] 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.

[0136] 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.

[0137] **DEFINITION**

[0138] 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.

[0139] 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 $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate.

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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.).

[0144] 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.

[0145] 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, C₂₋₆ 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.

[0146] 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, C₂₋₆ 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.

[0147] As used herein, "cycloalkyl" refers to a nonaromatic cyclic, bicyclic, 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.

[0148] 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.

[0149] 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, benzothieryl, benzofuranyl, isobenzofuran and the like.

[0150] 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 —NH₂, —NHR, or —NR₂, where R is the same or different at each occurrence and can be an alkyl group or an aryl group.

[0151] 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.

[0152] 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. Dihalalkyl 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.

[0153] As used herein, unless otherwise specified, the term "heteroatom" refers to a nitrogen (N), oxygen (O), or sulfur (S) atom.

[0154] **EXAMPLE**

[0155] **Example 1: Synthesis of exemplary compounds of the present disclosure**

[0156] ***Chemical Materials and Methods***

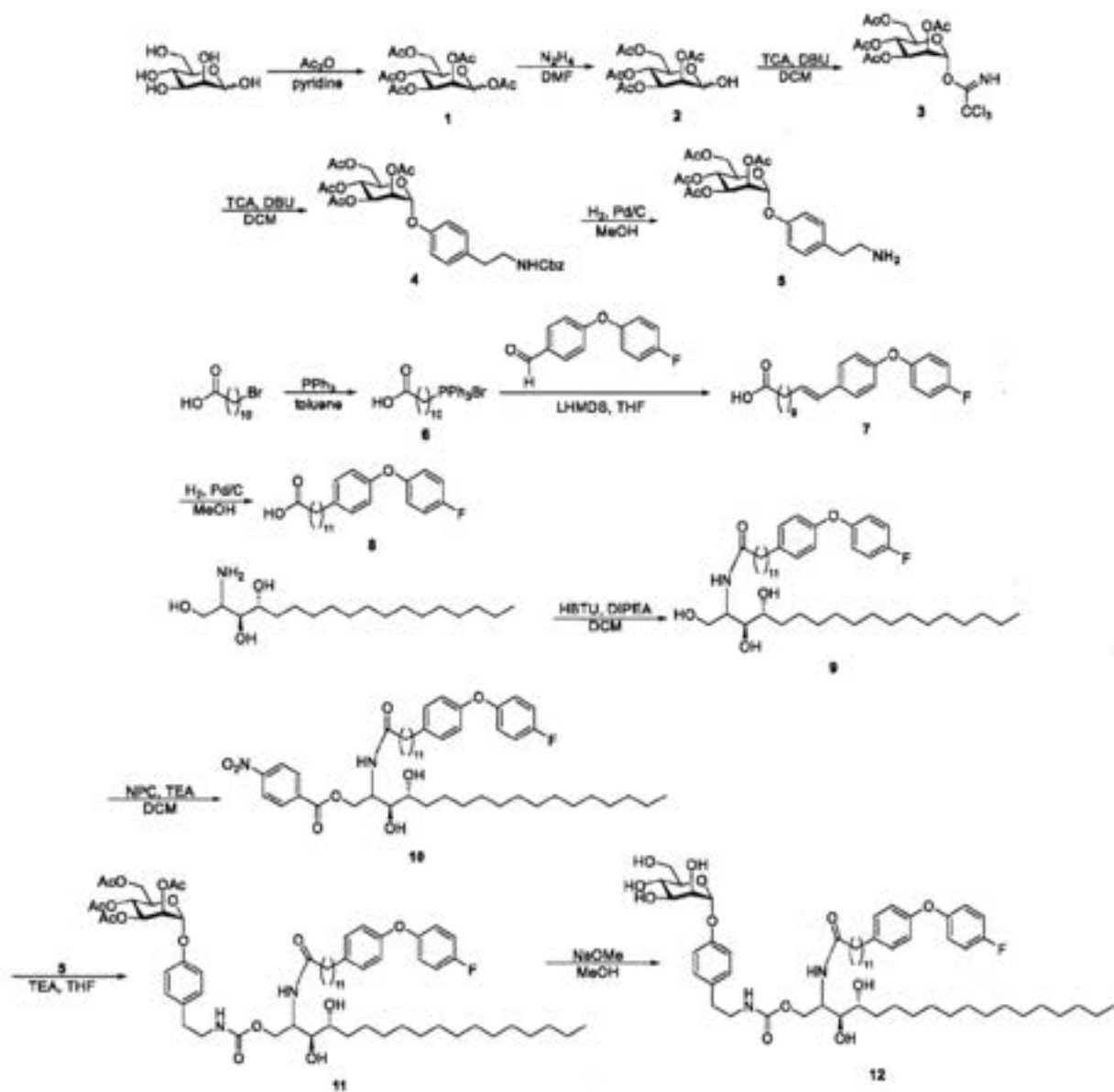
[0157] For chemical synthesis, all starting materials and commercially obtained reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry solvents. ^1H and ^{13}C NMR spectra were recorded on Bruker 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 $\text{DMSO}-d_6$ at δ 2.5 and 39.51 for ^1H 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. IMPACTTM 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.

[0158] The chemical materials and methods described herein apply to all examples described in the present disclosure.

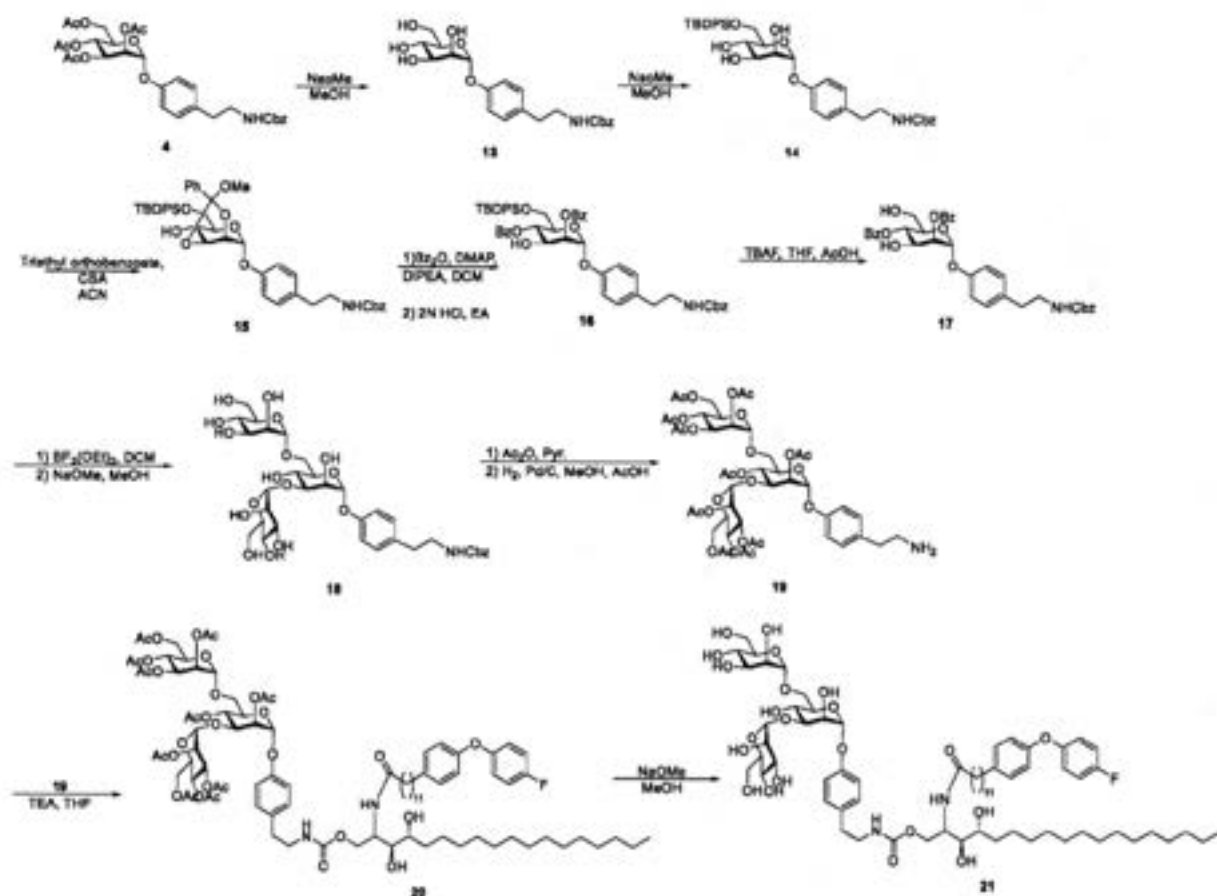
[0159] ***Synthesis and Results***

[0160] 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.

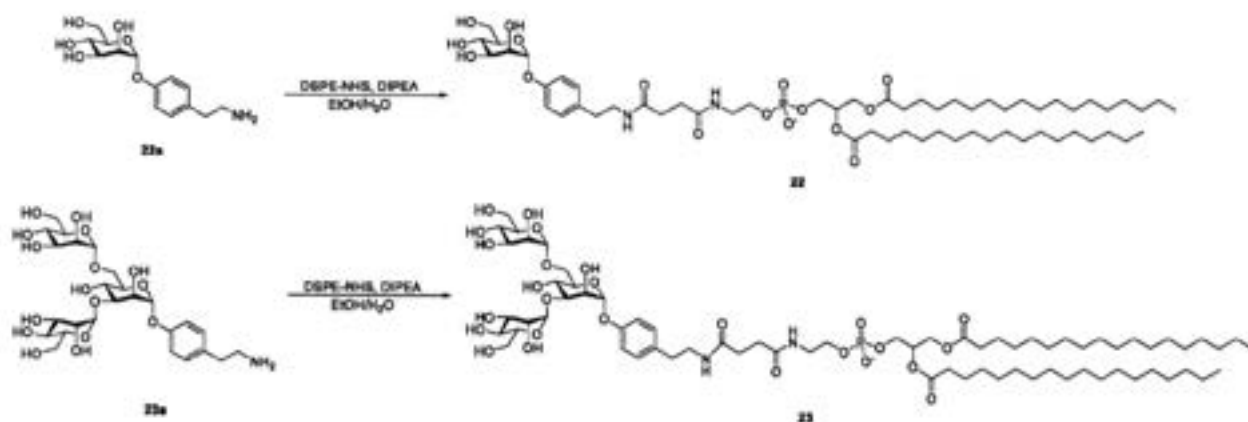
[0161] Scheme 1



[0162] Scheme 2



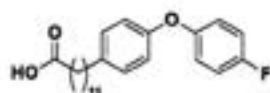
[0163] Scheme 3



[0164] Compounds 1 to 5

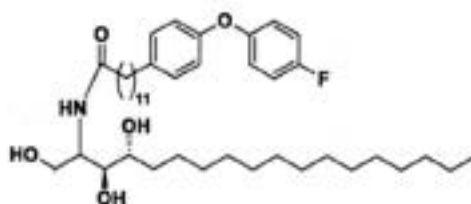
[0165] Compounds 1-5 were synthesized and characterized according to a published protocol (ACS Nano 2021, 15, 309–321).

[0166] Compound 8



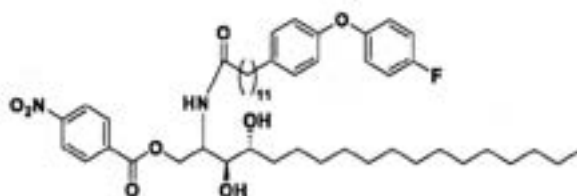
[0167] (11-Carboxynonyl)triphenylphosphonium bromide **6** (2.5 g, 10 mmol) was prepared by refluxing triphenylphosphine (10 mmol) and 11-bromoundecanoic acid (10 mmol). It was then dissolved in 50 ml of tetrahydrofuran (THF) and cooled to 0 °C. lithium bis(trimethylsilyl)amide (LHMDS; 1 M in THF, 20 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 MgSO₄. 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 H₂ 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%).

[0168] Compound 9



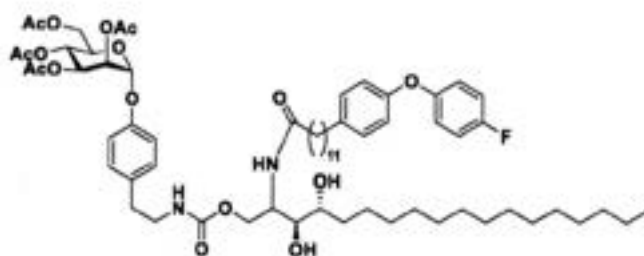
[0169] Compound **9**. Compound **8** (1 mmol) in THF (10 mL) was added EDC (1.5 mmol), HOBT (1.5 mmol), DMAP (0.1 mmol), trimethylamine (2 mmol), and phytosphingosine (1.2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1) to yield **9** (74%).

[0170] **Compound 10**



[0171] 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.

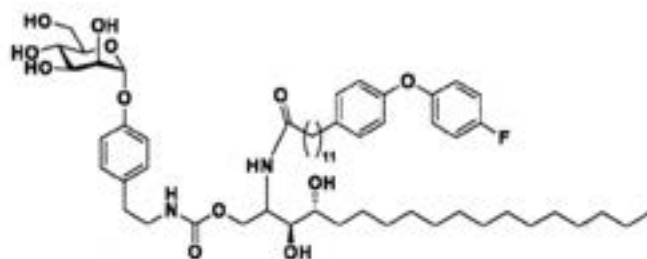
[0172] **Compound 11**



[0173] 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/H₂O. The collected organic layer was washed with

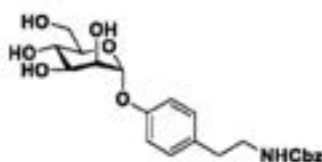
saturated $\text{NaHCO}_3(\text{aq})$, water and brine, and dried over MgSO_4 . The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **11** (59%).

[0174] **Compound 12**



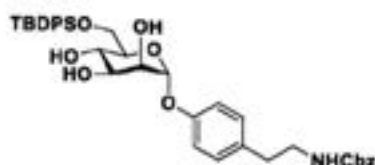
[0175] Compound **11** in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at room temperature for 2 hours. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo to give compound **12** (quant.).

[0176] **Compound 13**



[0177] 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.).

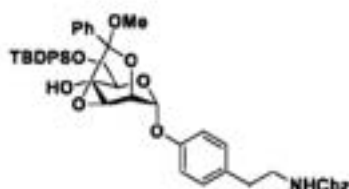
[0178] **Compound 14**



[0179] 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

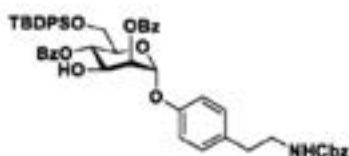
MeOH. After stirring at room temperature for 10 min, the solvent was removed under reduced pressure to give a dry residue that was purified by column chromatography with MeOH/DCM (1/10) to give compound **14** (82%).

[0180] **Compound 15**



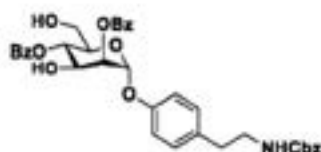
[0181] To a solution of compound **14** (1 mmol) and a catalytic amount of CSA (0.1 mmol) in CH₃CN (20 mL) was added trimethyl orthobenzoate (3 mmol) at room temperature under atmospheric pressure of nitrogen. After stirring for 30 min, Et₃N was added to quench the reaction, and the resulting mixture was dried under reduced pressure. The residue was purified by column chromatography with EA/Hex (1/2) to give compound **15** (79%).

[0182] **Compound 16**



[0183] 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/H₂O. The collected organic layer was washed with ice-cold saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **16** (71%).

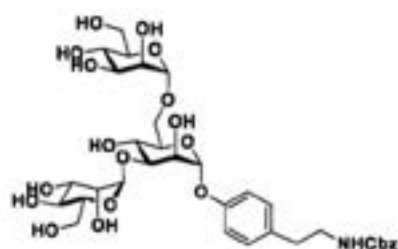
[0184] **Compound 17**



[0185] 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 NaHCO₃(aq), water, and brine, dried with anhydrous MgSO₄, and concentrated under reduced pressure. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **47** (88%).

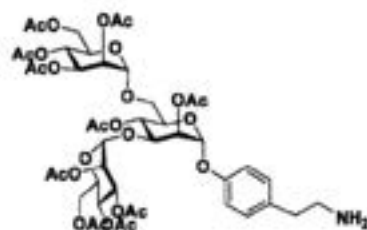
[0186]

[0187] **Compound 18**



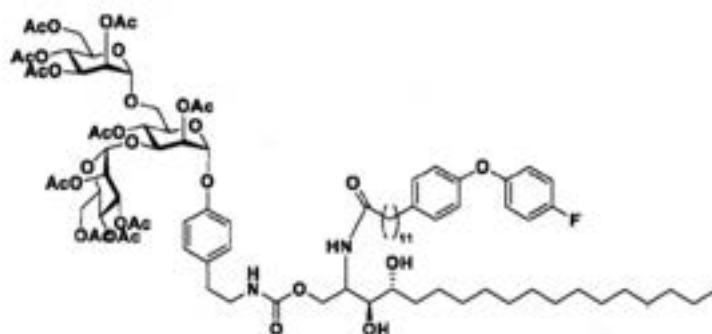
[0188] To a stirred solution of **17** (1 mmol) and 4 Å molecular sieve (0.1 g) in anhydrous DCM (10 mL) was cooled to -40°C and then BF₃(OEt)₂ (0.1 mmol) was added dropwise to the solution. A solution of **3** in anhydrous DCM was added dropwise to the above mixture and stirred for 1 h at -40°C. After 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. NaHCO₃ aq. and extracted with DCM. The organic layer was dried with MgSO₄ 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 H₂O as eluent to obtain a pure trisaccharide. The compound was lyophilized to dryness to give compound **18** (39%).

[0189] **Compound 19**



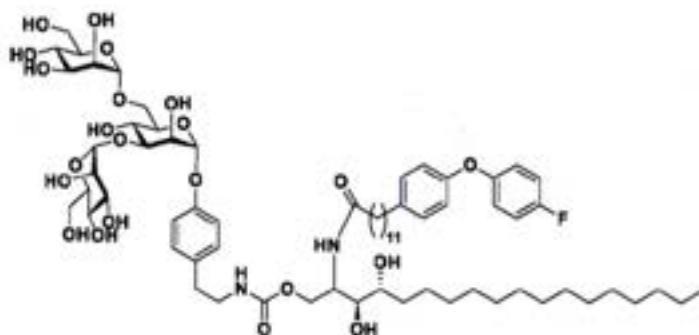
[0190] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo.

[0191] **Compound 20**

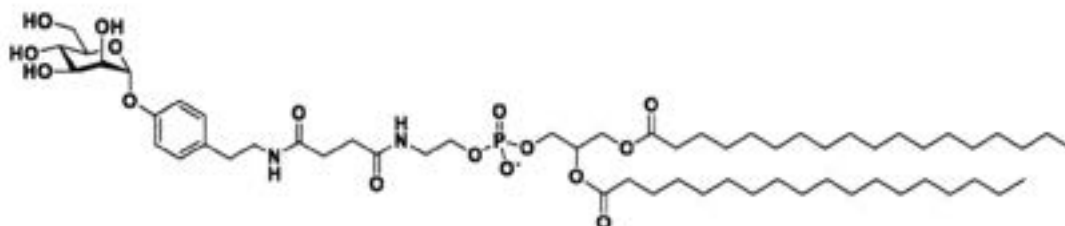


[0192] Compound **19** (1 mmol) in THF (10 mL) was added **10** (1 mmol) and trimethylamine (2 mmol), and the resulting solution was stirred under nitrogen at rt for 2 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **20**.

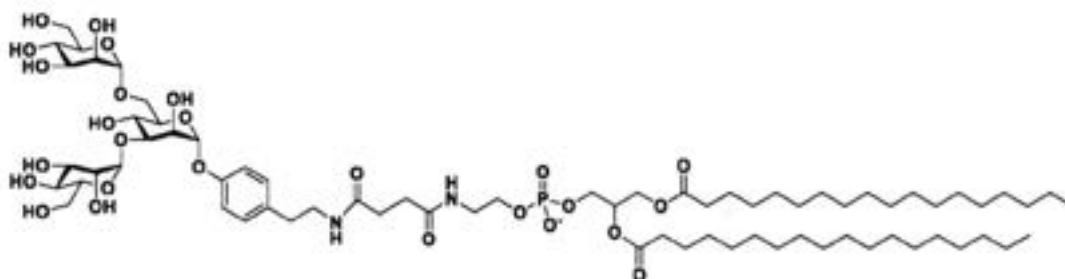
[0193] **Compound 21**



[0194] 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).

[0195] **Compound 22**

[0196] Arylmannoside **22s** (0.1 mmol) in EtOH/H₂O (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 H₂O as eluent to yield **22** (79%).

[0197] **Compound 23**

[0198] Aryltrimannoside **23s** (0.1 mmol) in EtOH/H₂O (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 H₂O as eluent to yield **23** (76%).

[0199] **Example 2: Preparation and characterization of the LNP of the present disclosure**[0200] **Preparation of LNP**

[0201] 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.

[0202] **Characterization of LNP**

[0203] **Size Measurement.** The LNP prepared above was examined using dynamic light scattering (DNP) to measure its size. First, 5 μ L of the LNP solution was transferred to a clean 1.5 mL tube and diluted with 95 μ L 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.

[0204] Table: the size and PDI measurement

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

[0205] **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 μ L) was prepared to obtain a standard curve. 5 μ L of the LNP solution prepared above was transferred to a clean tube and diluted to a final volume of 100 μ L. The working solution of the quant-it Ribogreen reagent (100 μ L) 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.

[0206] Table: Zeta potential and encapsulation efficiency

Sample	Zeta potential (mV)	Encapsulation efficiency (%)
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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

[0207] **Example 3: *In vitro* uptake and transfection of mRNA-LNPs in Dendritic cells.**

[0208] *Experiment 3-1*

[0209] *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 μ M 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 μ M 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.

[0210] 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

[0211] *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 °C 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).

[0212] *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).

[0213] *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-BMDCs, 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.

[0214] 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

[0215] *Experiment 3-2*

[0216] 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, 1x10⁶ 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.

[0217] 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°C for 48 hours. The experiments were conducted using FACS, similar to that described above.

[0218] 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

[0219] *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.

[0220] 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

[0221] **Experiment 3-3**

[0222] 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 CaCl₂, 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 tetramethylbenzidine (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 K_d was calculated using a nonlinear regression curve fit for total binding using GraphPad Prism.

[0223] **Example 4: *In vivo* delivery of luciferase mRNA-LNP**

[0224] 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 CO₂ 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.

[0225] 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

[0226] *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.

[0227] **Example 6: Immunization**

[0228] *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).

[0229] *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.

[0230] *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.

[0231] *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- $\Delta\text{R8.91}$, 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 $\times g$ 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 AlamarBlue 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 titrating pseudotyped lentivirus, different amounts of lentivirus were added into the culture medium containing polybrene (final concentration 8 $\mu\text{g}/\text{ml}$). Spin infection was carried out at 1,100 $\times g$ 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 $\mu\text{g}/\text{ml}$ 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 $(RLU^{control} - RLU^{Serum})/RLU^{control}$.

[0232] *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 H₂SO₄. After incubation for 30 minutes, absorbance (OD 450 nm) was measured using SpectraMax M5.

[0233] *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.

[0234] **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.

[0235] **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.

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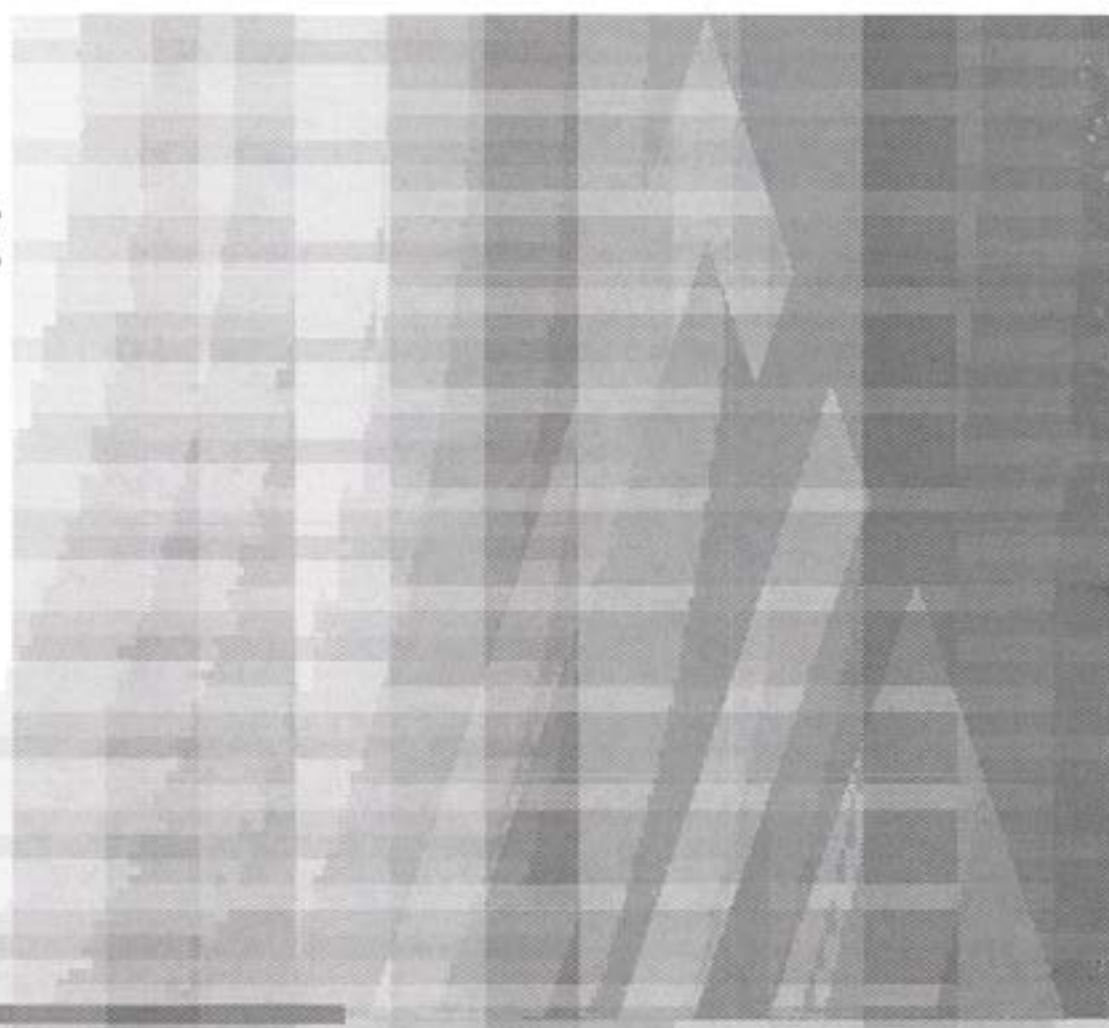
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Rock BioMedical, Inc.



NOVEL DENDRITIC CELL TARGETING

mRNA VACCINE FORMULATIONS

09/30/2023

CONFIDENTIAL

About Us - Rock BioMedical

Founded in 2021, Rock BioMedical, Inc. (Rock Bio) is a biopharmaceutical company seeking to combine both active immunization (eg vaccines) and passive immunization (eg antibodies) for the eradication of diseases based on our proprietary Glyco-engineered Universal Vaccine platform.

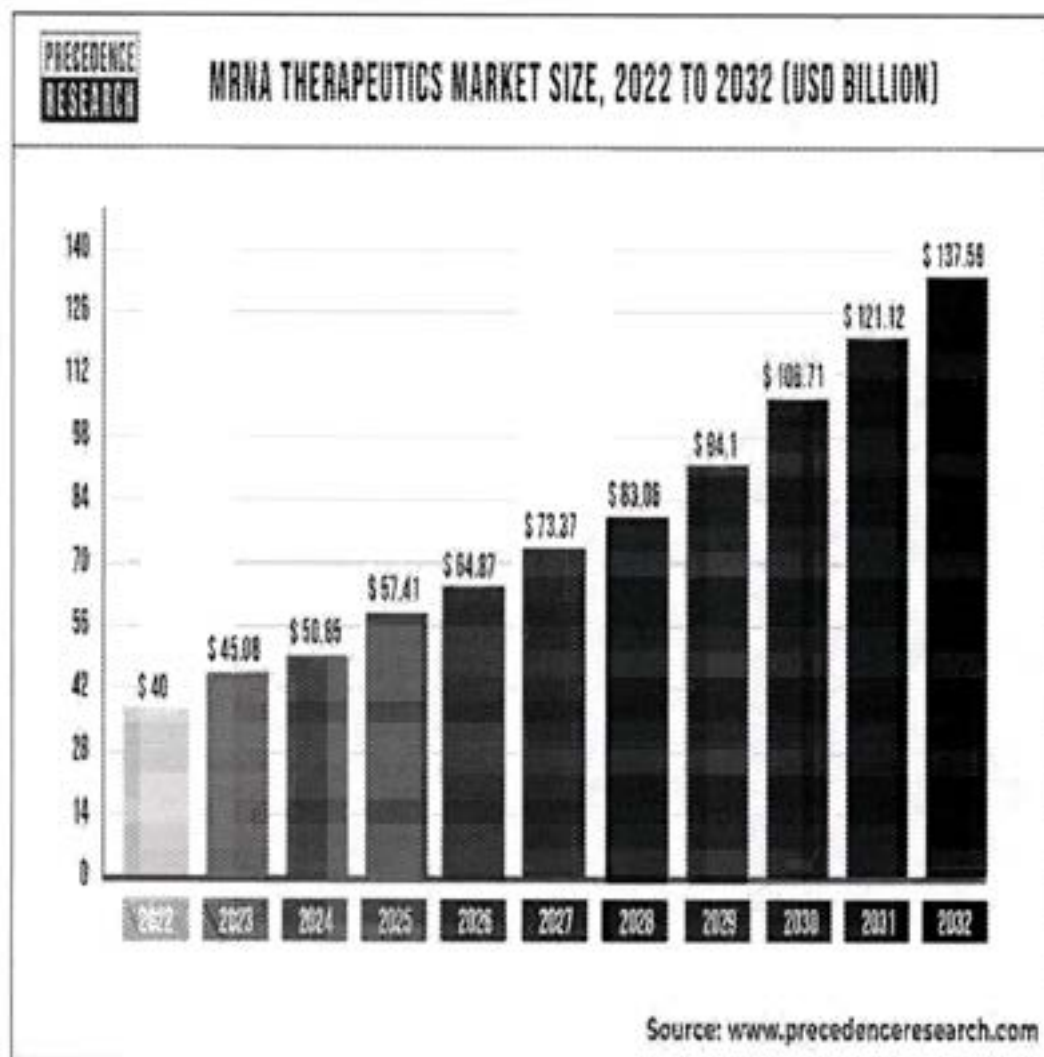


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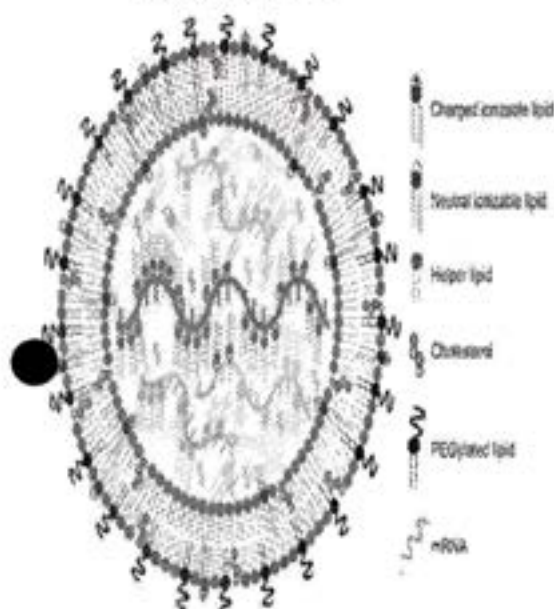
Taipei, Taiwan

Rising Market for mRNA Therapeutics



LNP components for mRNA vaccine formulations

Lipid Nanoparticle (LNP)

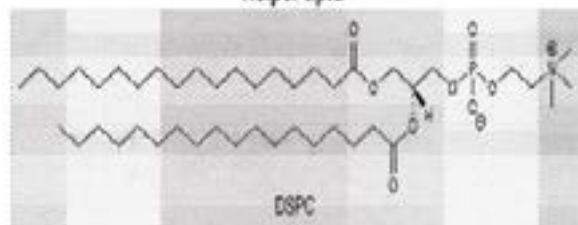


Confirmed or Probable Ionizable lipid

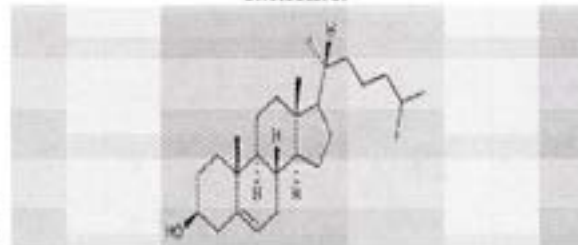
Molar lipid ratios (%)^a

Lipid H, SM-102 (mRNA-1273)	Moderna nucleoside modified mRNA	50 : 10 : 38.5 : 1.5
Aclaris ALC-0315 (BNT162b2)	BioNTech nucleoside modified mRNA	46.3 : 9.4 : 42.7 : 1.5

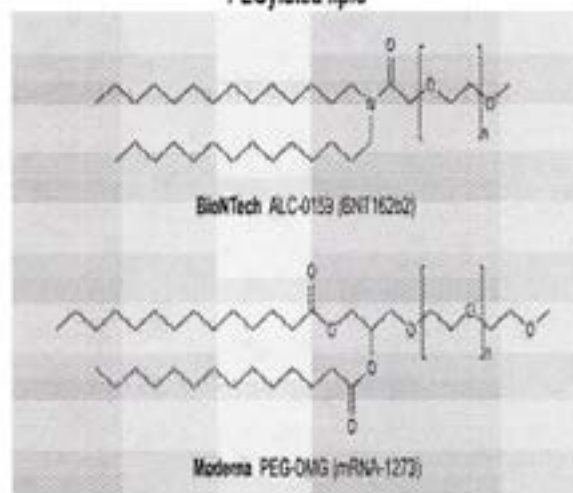
Helper lipid



Cholesterol



PEGylated lipid



Signal Transduct. Target Ther. 2022, 7, 94

Challenges of current mRNA vaccine development

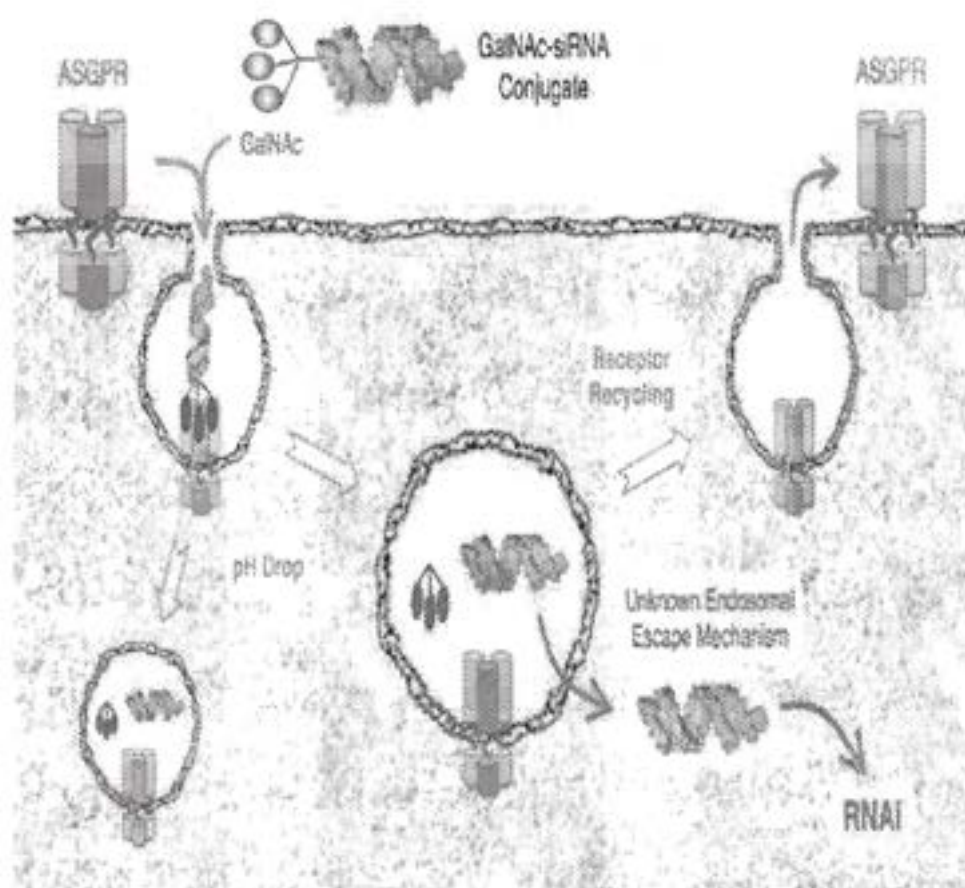
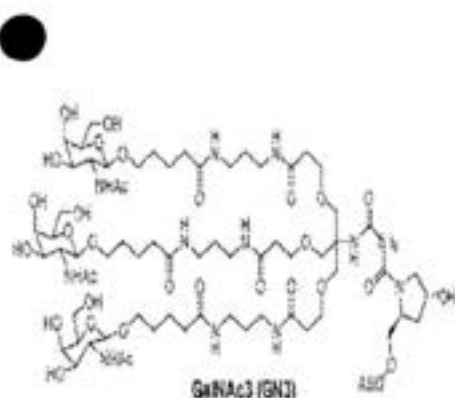
- Complicated IP landscape
- Lack of specific cell targeting capacity
- Safety and adverse effects
- Cold chain storage requirement

Development of novel mRNA vaccine formulations with specific cell targeting capacity

- mRNA encapsulation
- *In vivo* stability
- Specific cell targeting
- Internalization
- mRNA cargo release
- Biodegradable
- New IP

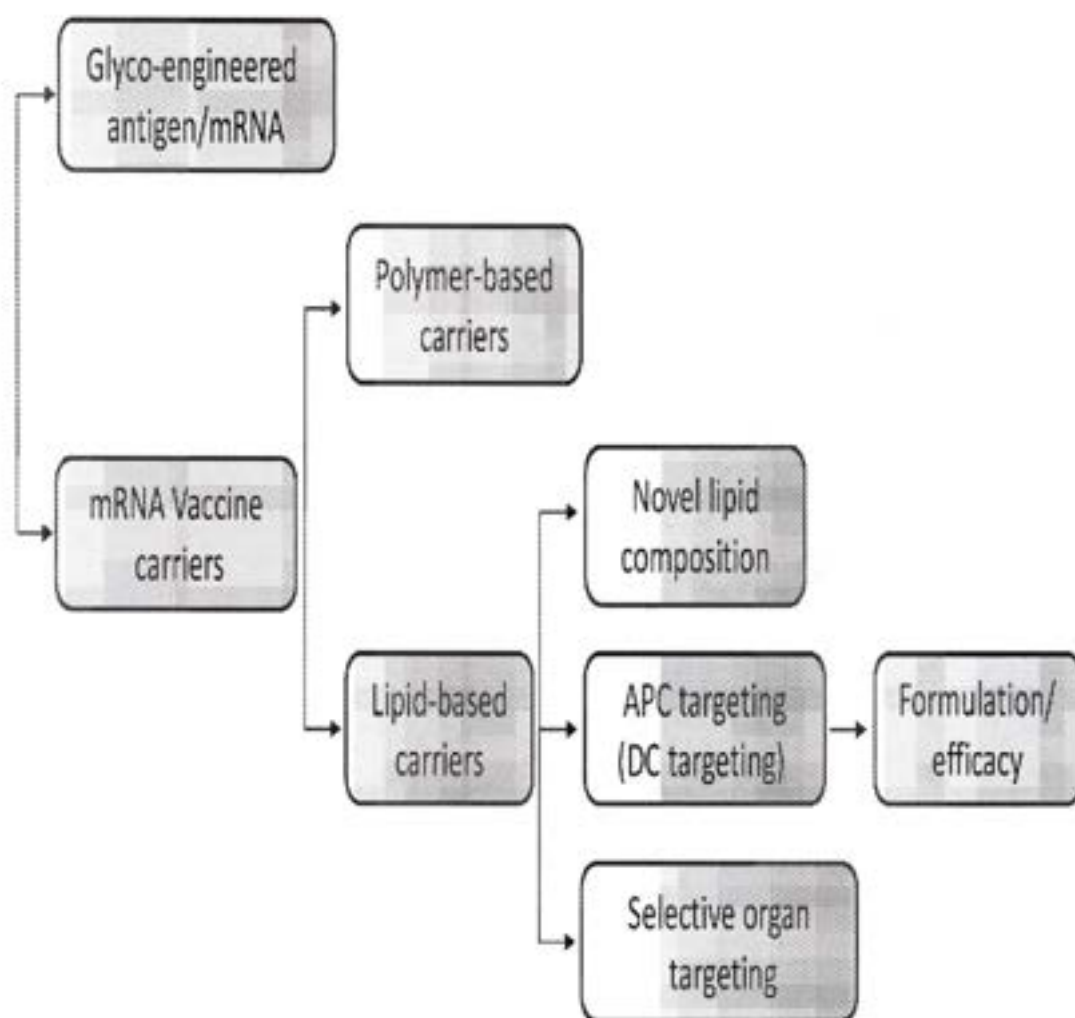
Targeted delivery via glycan-protein interaction

Targeting ASGPR via Tri-GalNAc

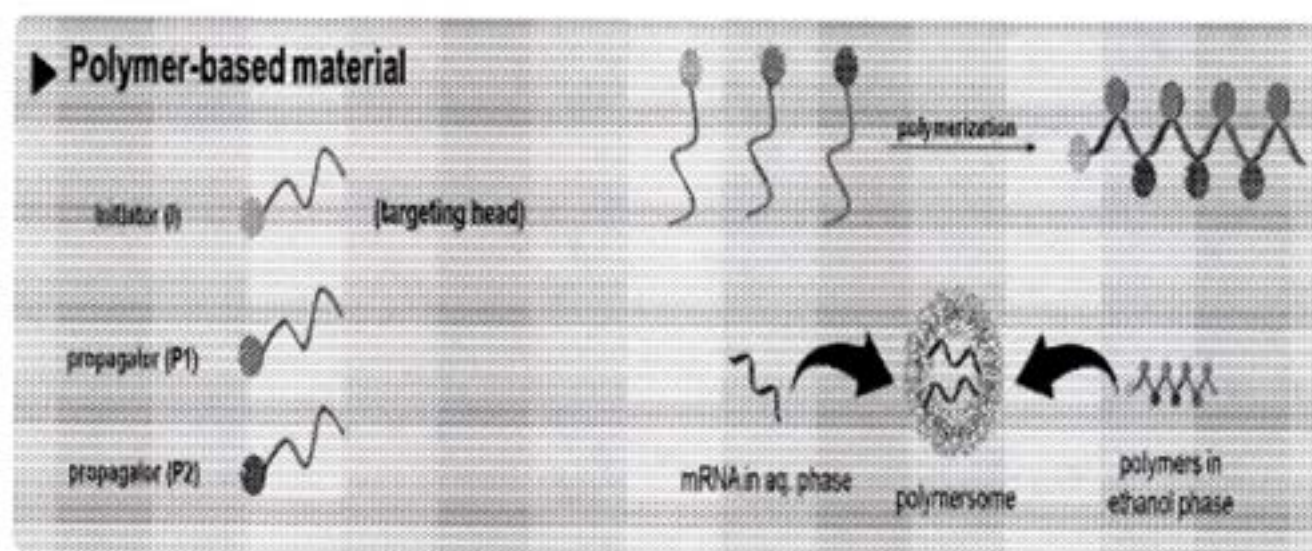


ACS Omega **2021**, *6*, 16259–16265

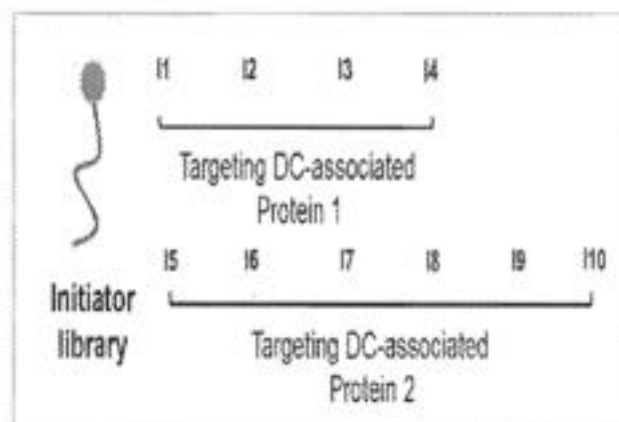
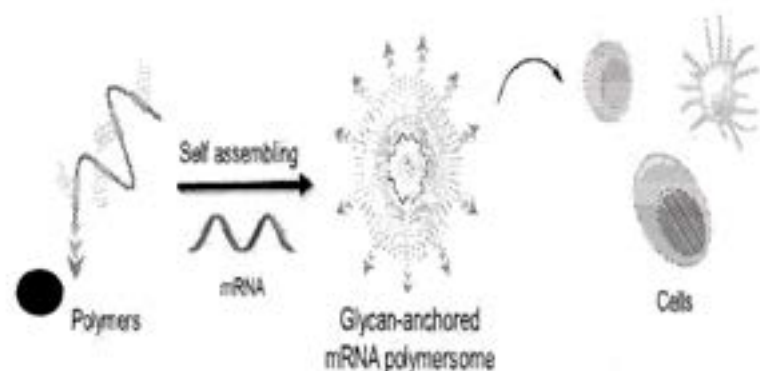
Rock BioMedical's Universal Vaccine Platform



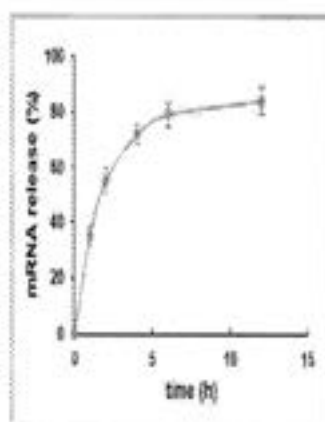
Carrier materials for novel mRNA vaccine formulations



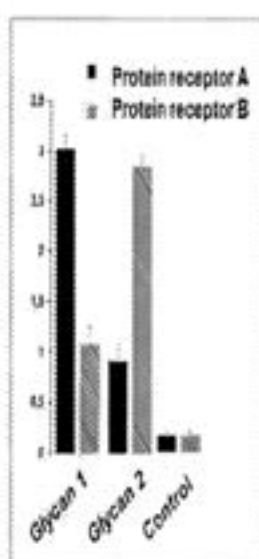
Novel polymers for encapsulation and targeted delivery of mRNA



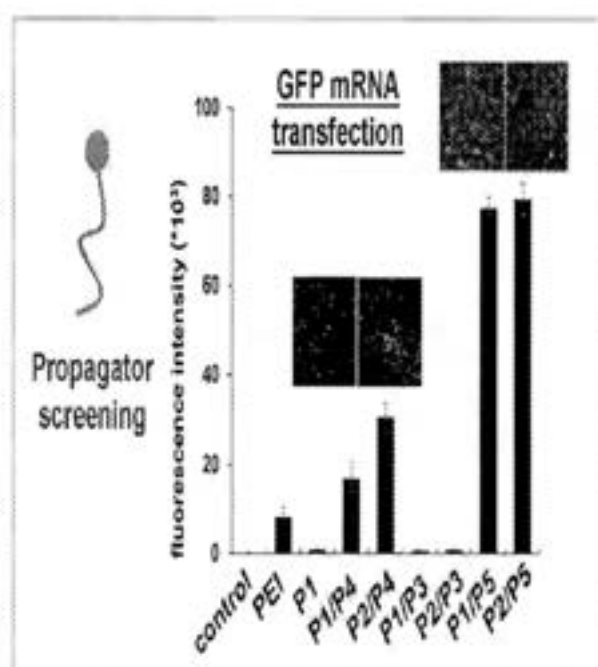
TEM image



mRNA release



Glycan-protein binding



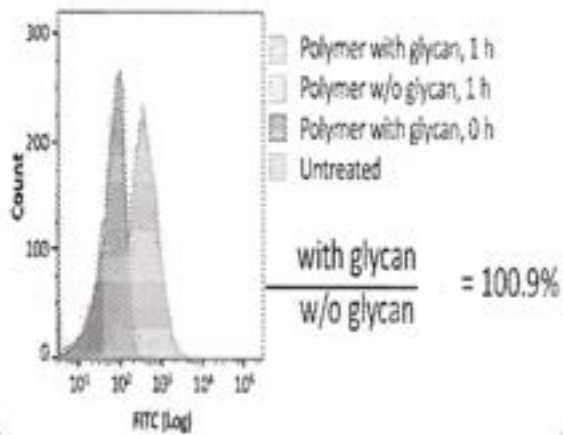
Propagator screening

Glycan conjugated polymersome yields enhanced uptake by dendritic cells and improved immune response

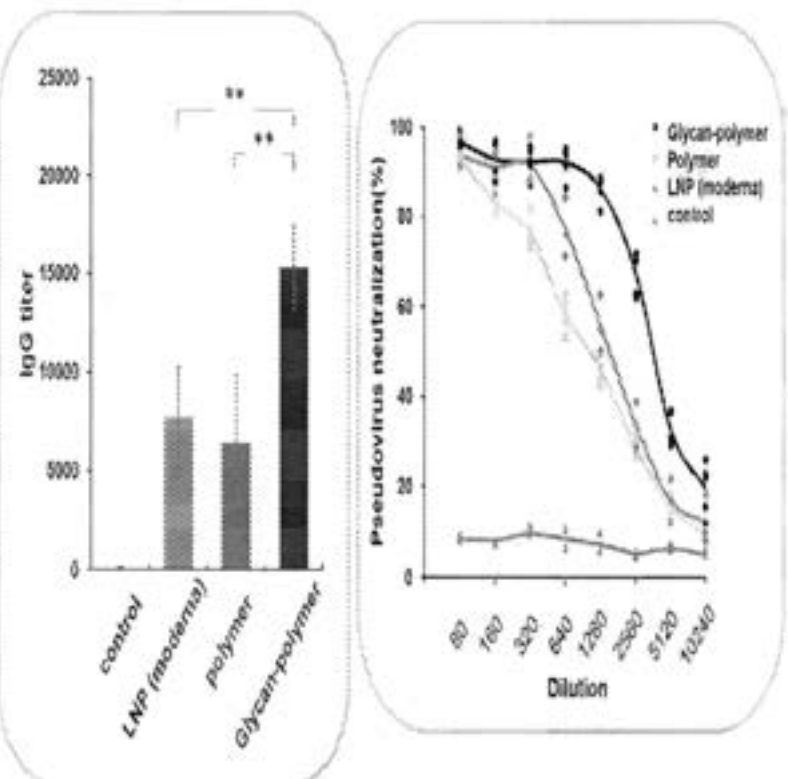
Uptake by BMDCs

condition	uptake ratio (with glycan / w/o glycan) (%)
A	140 ± 10
B	155 ± 25
C	115 ± 10
D	125 ± 20

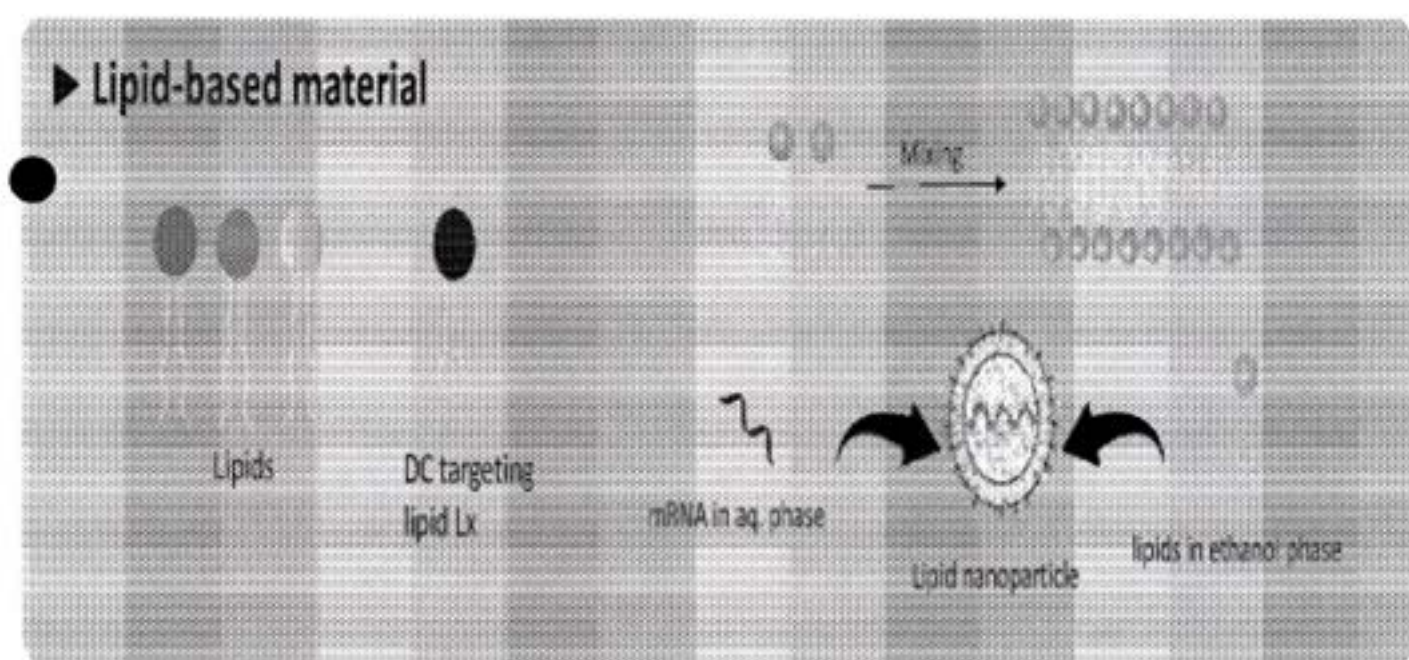
C2C12 muscle cell uptake



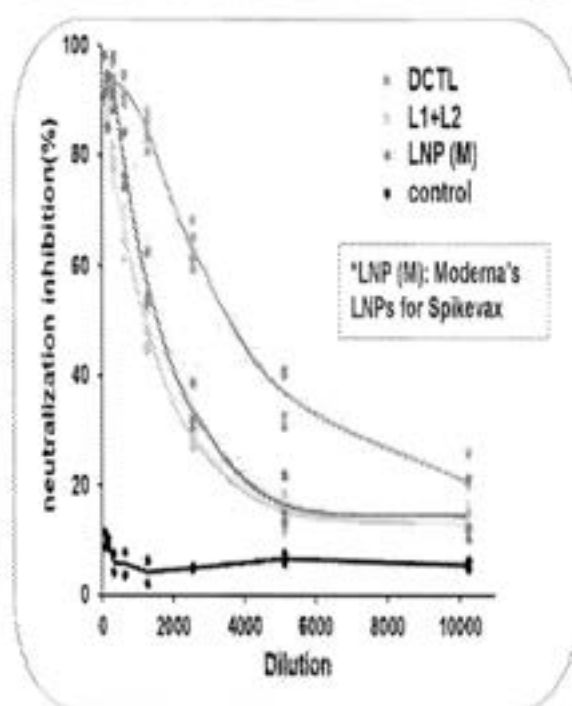
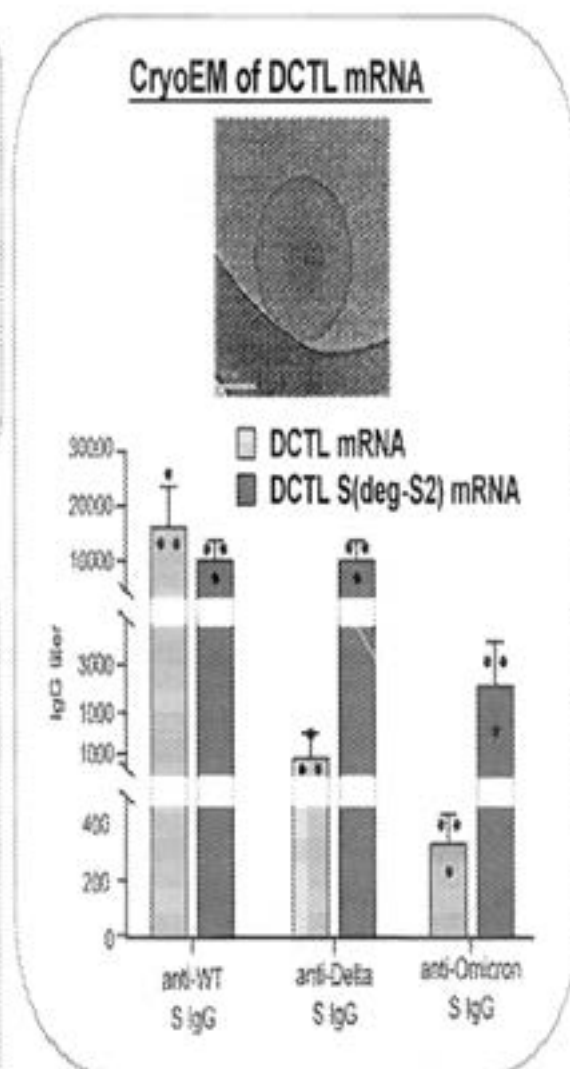
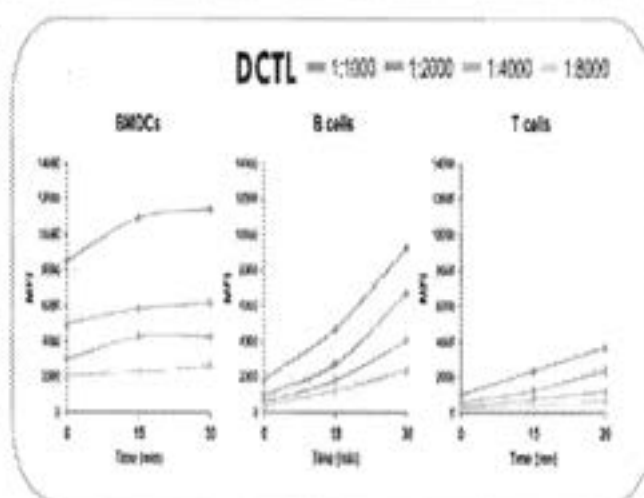
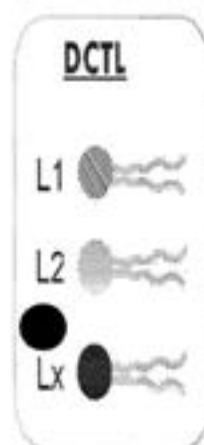
Assessment of Immunogenicity



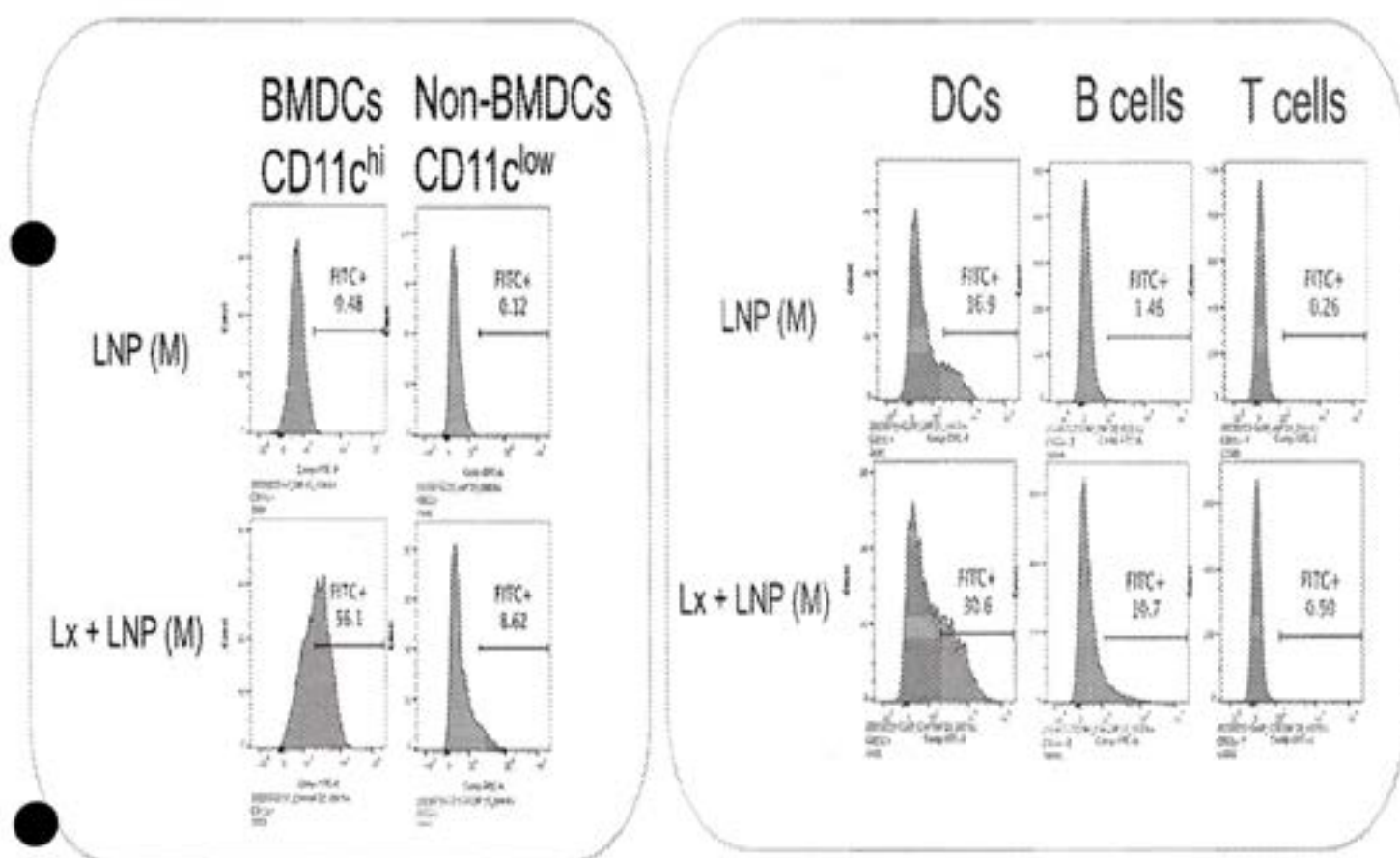
Carrier materials for novel mRNA vaccine formulations



Novel dendritic cell targeting LNPs (DCTL) yields enhanced uptake by DC and improved immune response

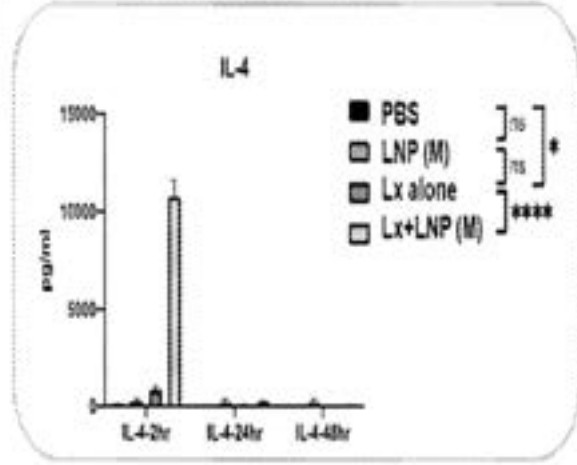
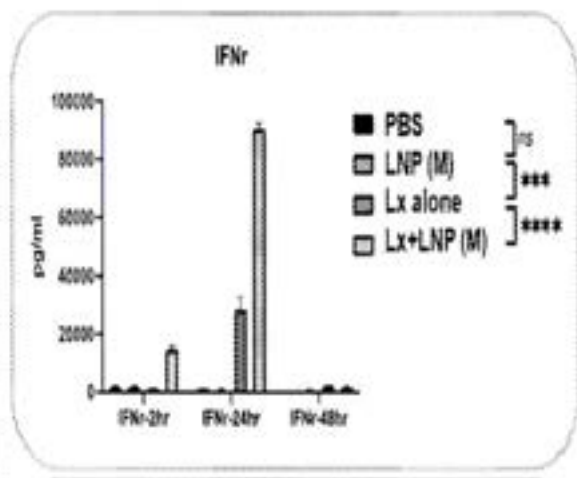
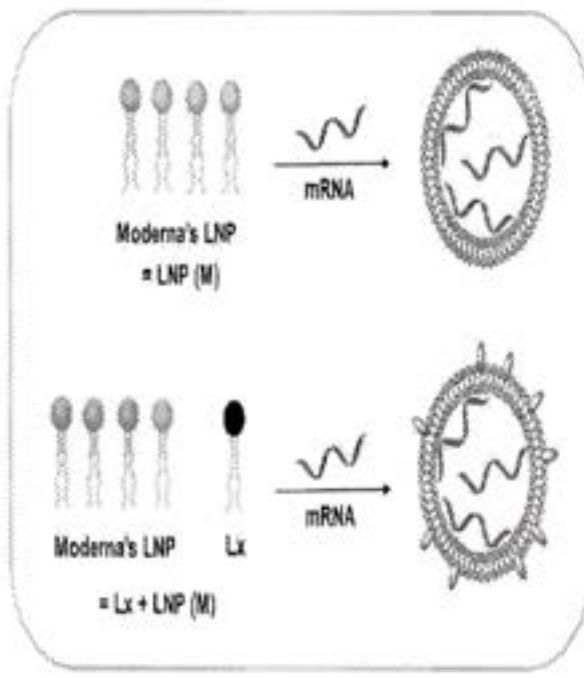
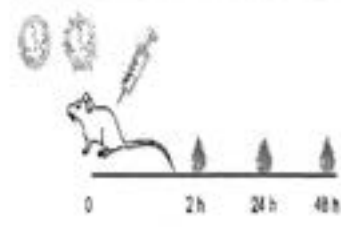


Incorporation of DC targeting lipid Lx improved LNP uptake by BMDCs

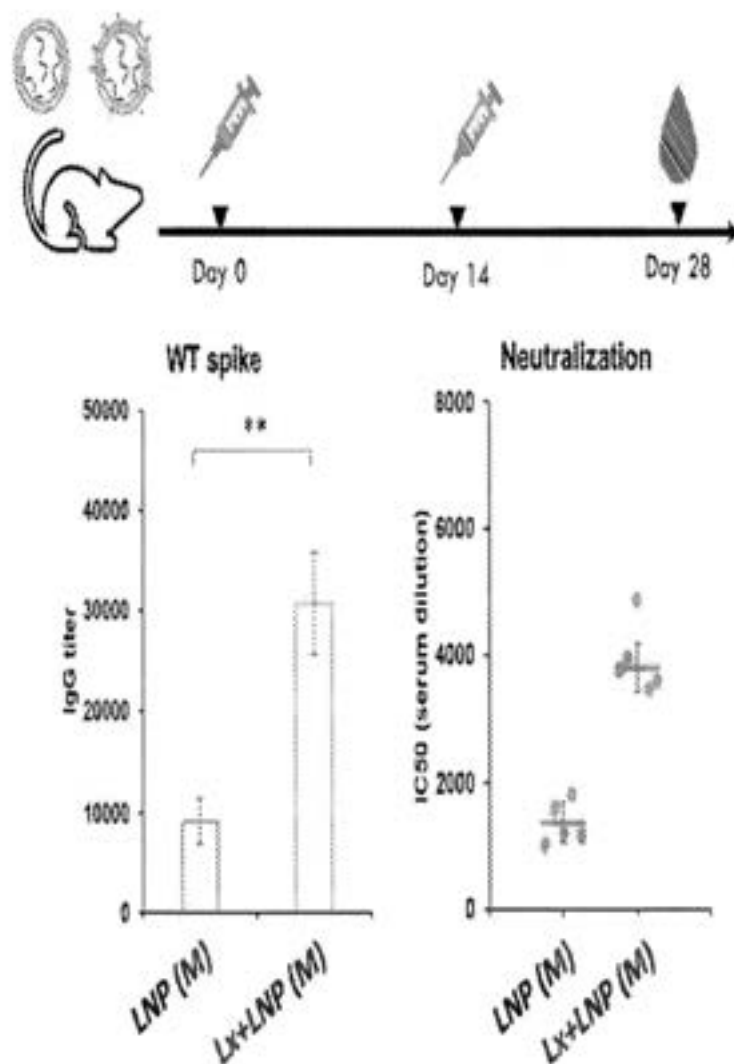


*BMDCs: bone marrow derived dendritic cells; LNP (M): Moderna's LNPs for Spikevax; Lx: DC targeting lipid

Incorporation of DC targeting lipid Lx improved cytokine responses after immunization



Incorporation of DC targeting lipid Lx improved humoral response after immunization



Summary

- Glycan-protein interaction represents a major way in which glycan structures mediate biological activities. By glycan engineering on both polymer- and lipid-based materials, important breakthrough has been made in our development of novel dendritic cell targeting mRNA vaccine formulations.
- A glycan conjugated lipid Lx effectively enhanced uptake by dendritic cells and improved immune response either as a component of a three-lipid proprietary LNP formulation, or as an additive to the approved LNP formulation used by Moderna's Spikevax.
- The glycan engineering platform can be expanded for mRNA delivery to other cell types for future development.

Thank you for your attention

For collaboration/partnership, please contact:

● Jeng-Shin Lee, MD, PhD

· +886-2-2788-5808 ext 111

· jslee@rockbiomedical.com.tw

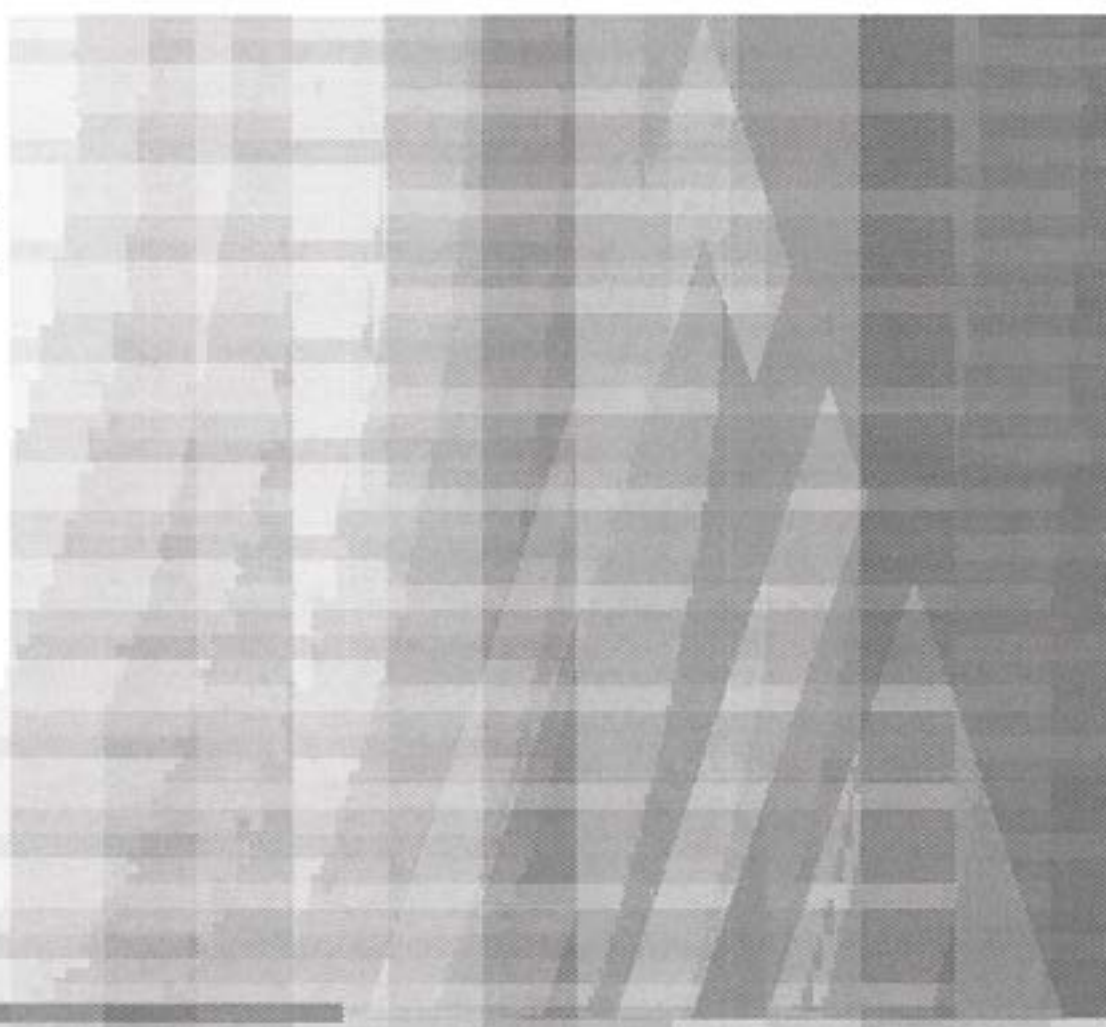
· <http://www.rockbiomedical.com.tw>

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● Taipei City 115202, Taiwan



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Rock BioMedical, Inc.



NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS

09/30/2023

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About Us - Rock BioMedical

Founded in 2021, Rock BioMedical, Inc. (Rock Bio) is a biopharmaceutical company seeking to combine both active immunization (eg vaccines) and passive immunization (eg antibodies) for the eradication of diseases based on our proprietary Glyco-engineered Universal Vaccine platform.

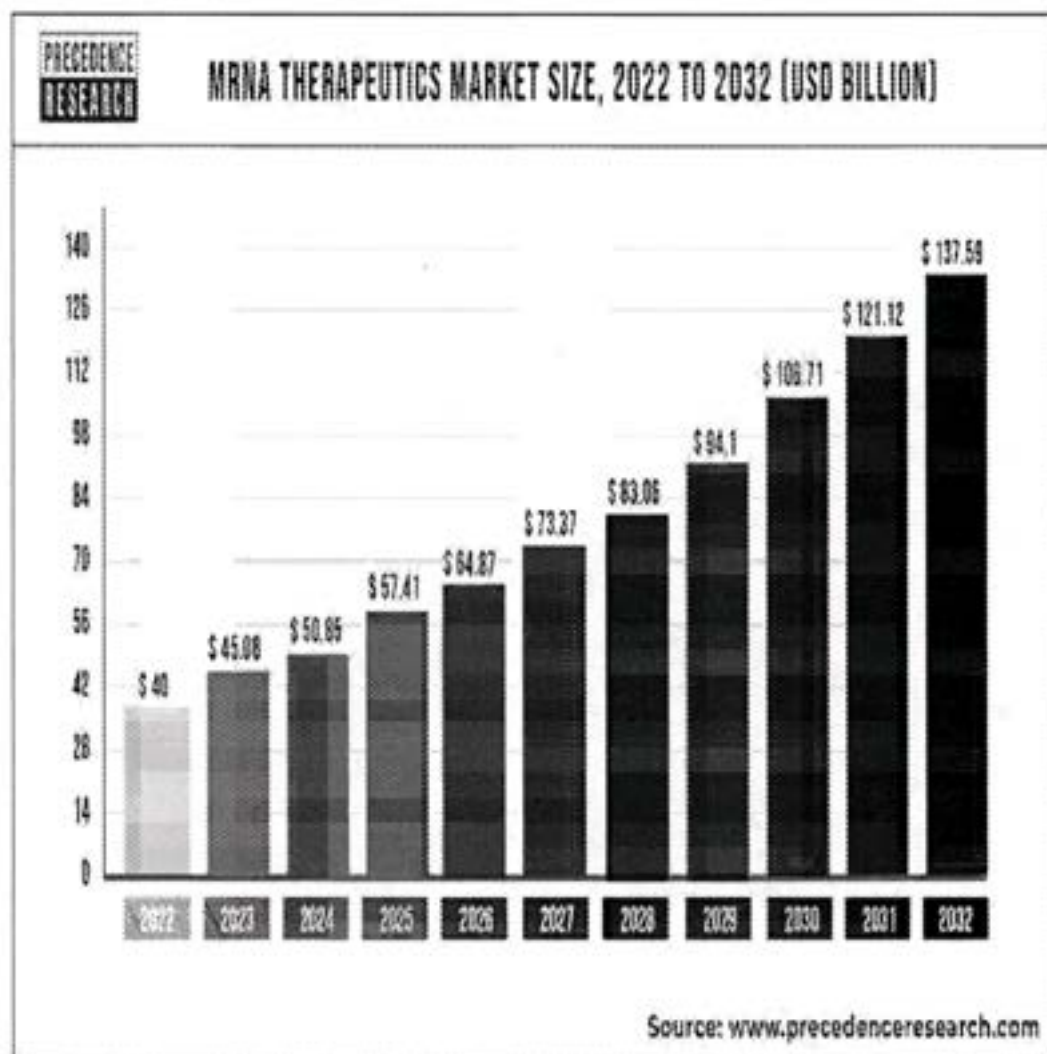


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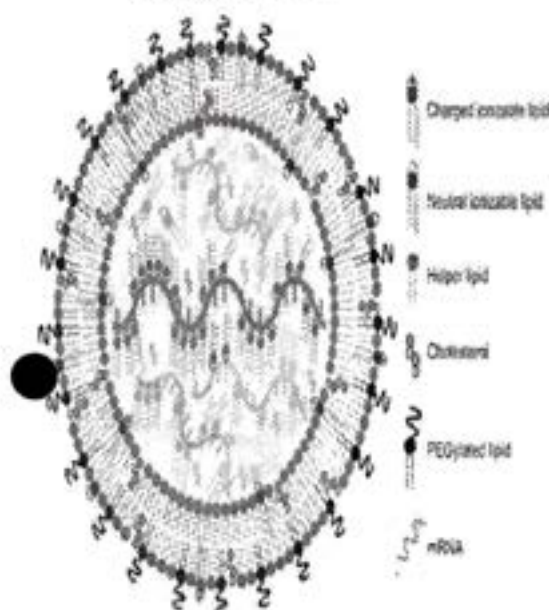
Taipei, Taiwan

Rising Market for mRNA Therapeutics



LNP components for mRNA vaccine formulations

Lipid Nanoparticle (LNP)

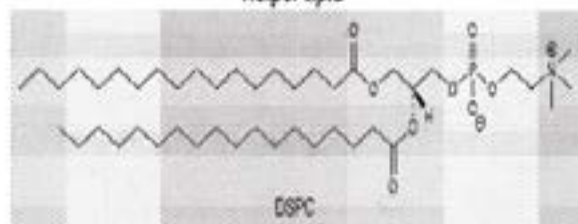


Confirmed or Probable Ionizable lipid

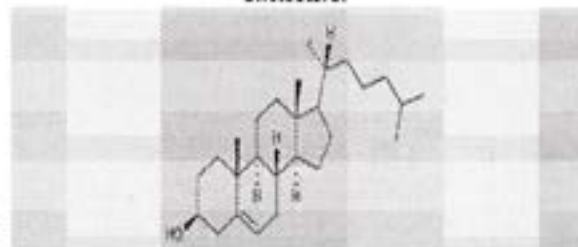
Molar lipid ratios (%)^a

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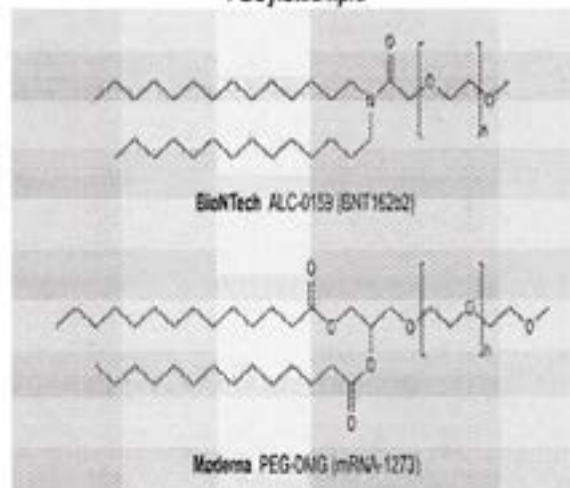
Helper lipid



Cholesterol



PEGylated lipid



Signal Transduct. Target Ther. 2022, 7, 94

Challenges of current mRNA vaccine development

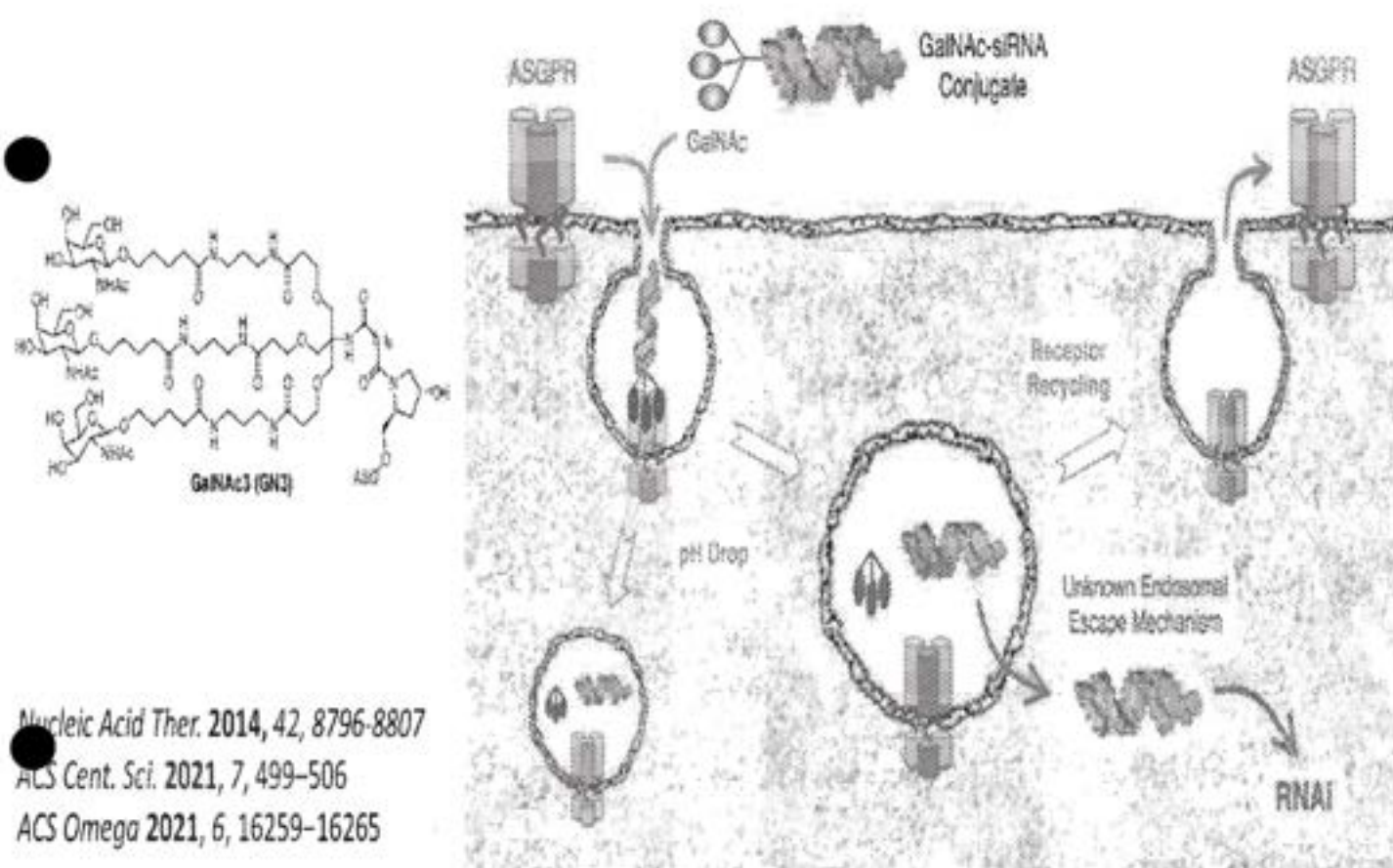
- Complicated IP landscape
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Development of novel mRNA vaccine formulations with specific cell targeting capacity

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- New IP

Targeted delivery via glycan-protein interaction

Targeting ASGPR via Tri-GalNAc

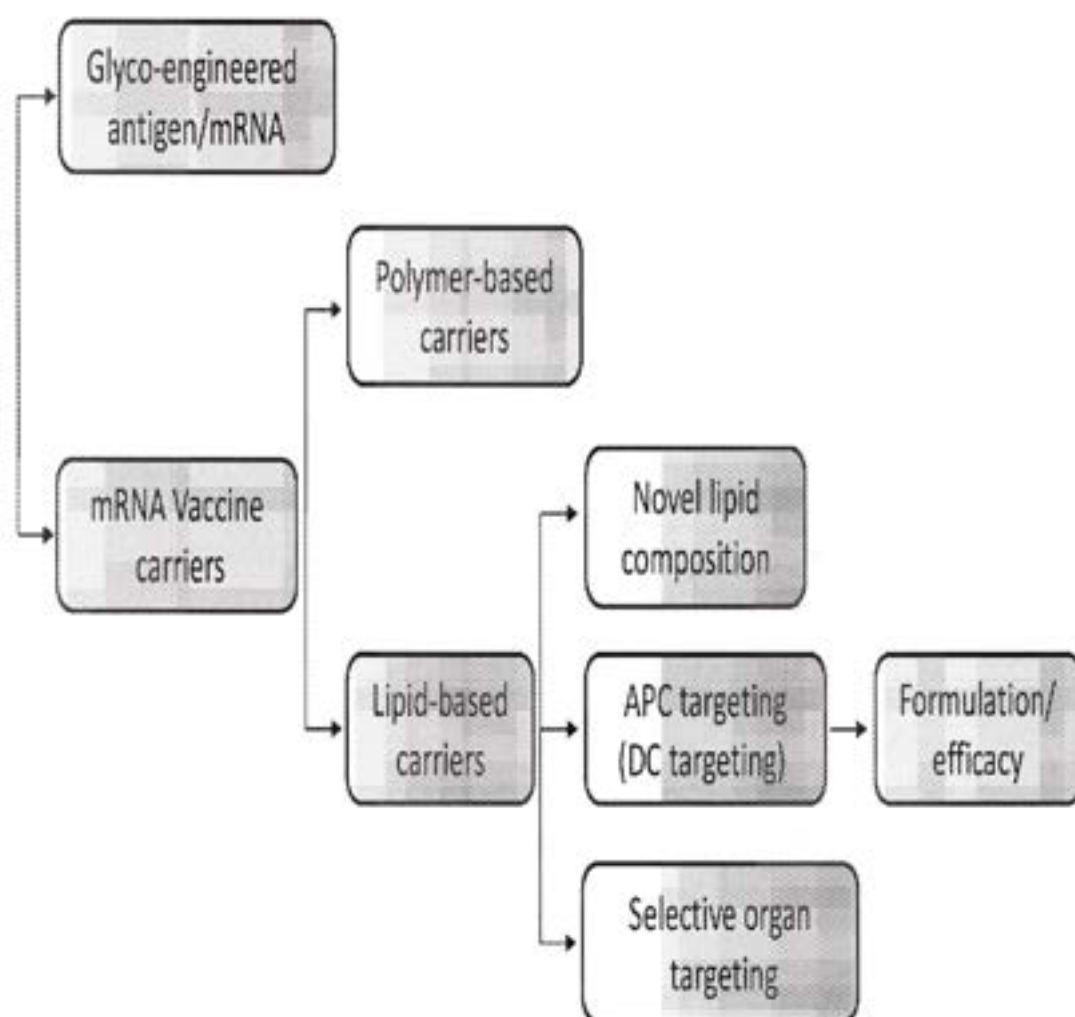


Nucleic Acid Ther. 2014, 42, 8796-8807

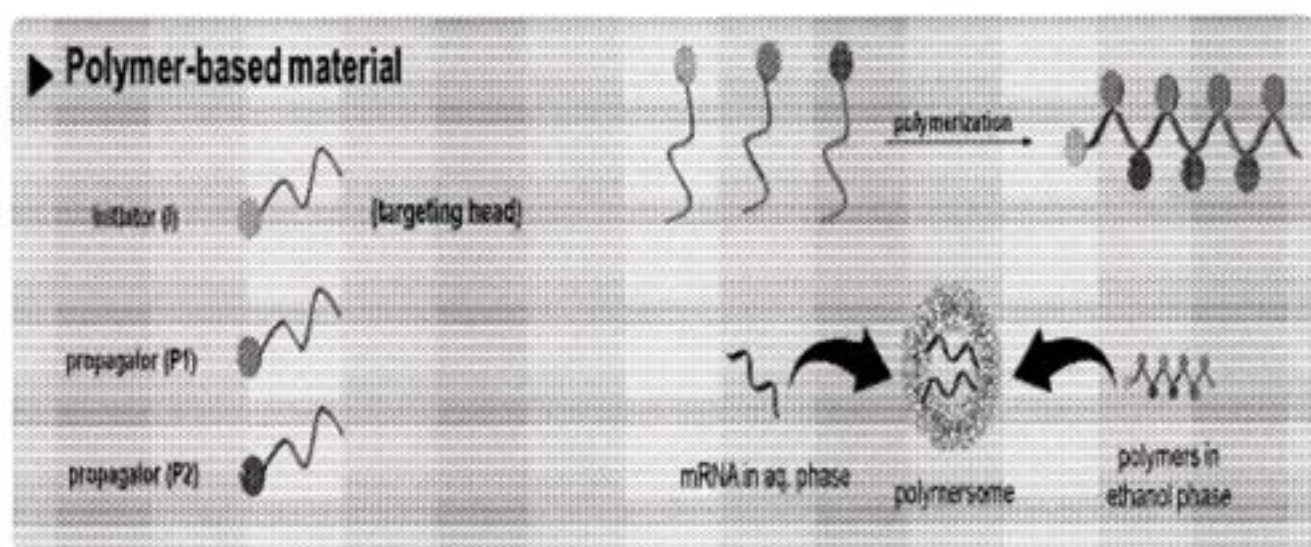
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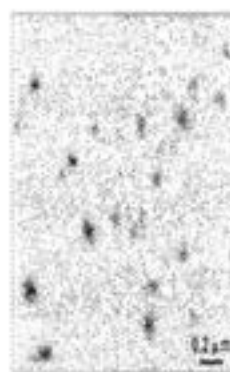
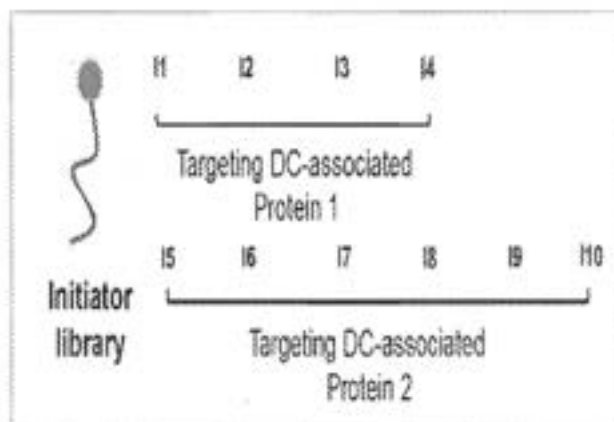
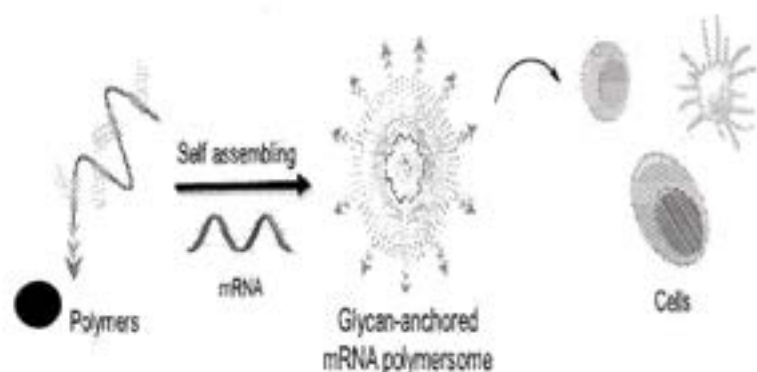
Rock BioMedical's Universal Vaccine Platform



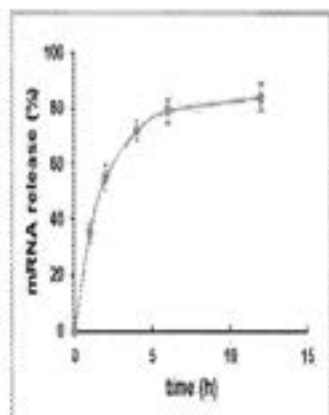
Carrier materials for novel mRNA vaccine formulations



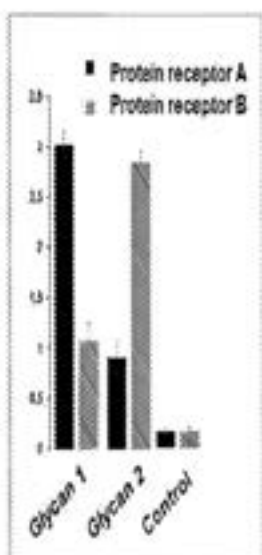
Novel polymers for encapsulation and targeted delivery of mRNA



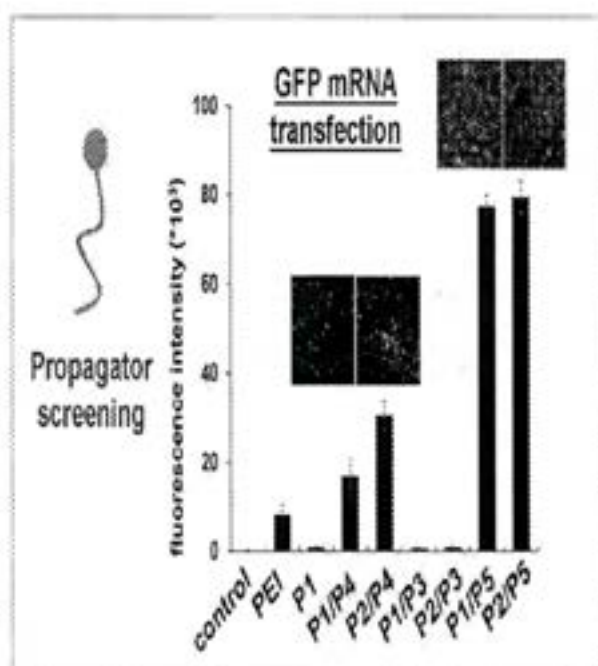
TEM image



mRNA release



Glycan-protein binding

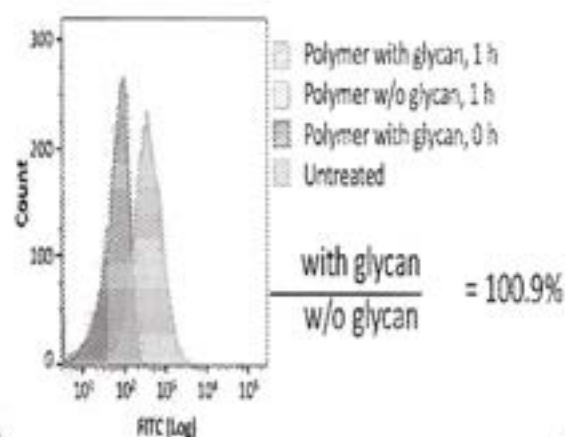


Glycan conjugated polymersome yields enhanced uptake by dendritic cells and improved immune response

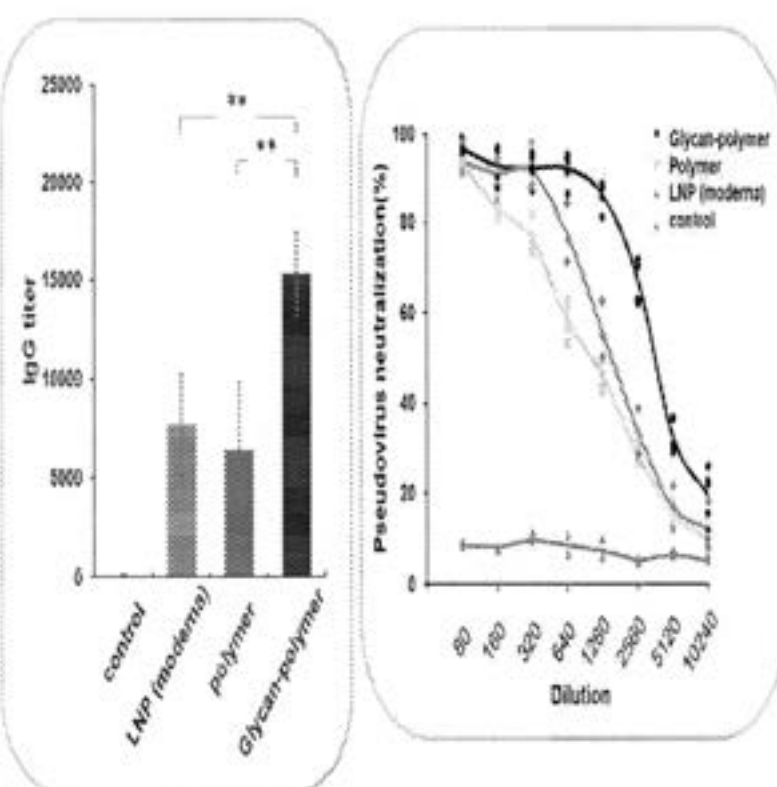
Uptake by BMDCs

condition	uptake ratio (with glycan / w/o glycan) (%)
A	140 ± 10
B	155 ± 25
C	115 ± 10
D	125 ± 20

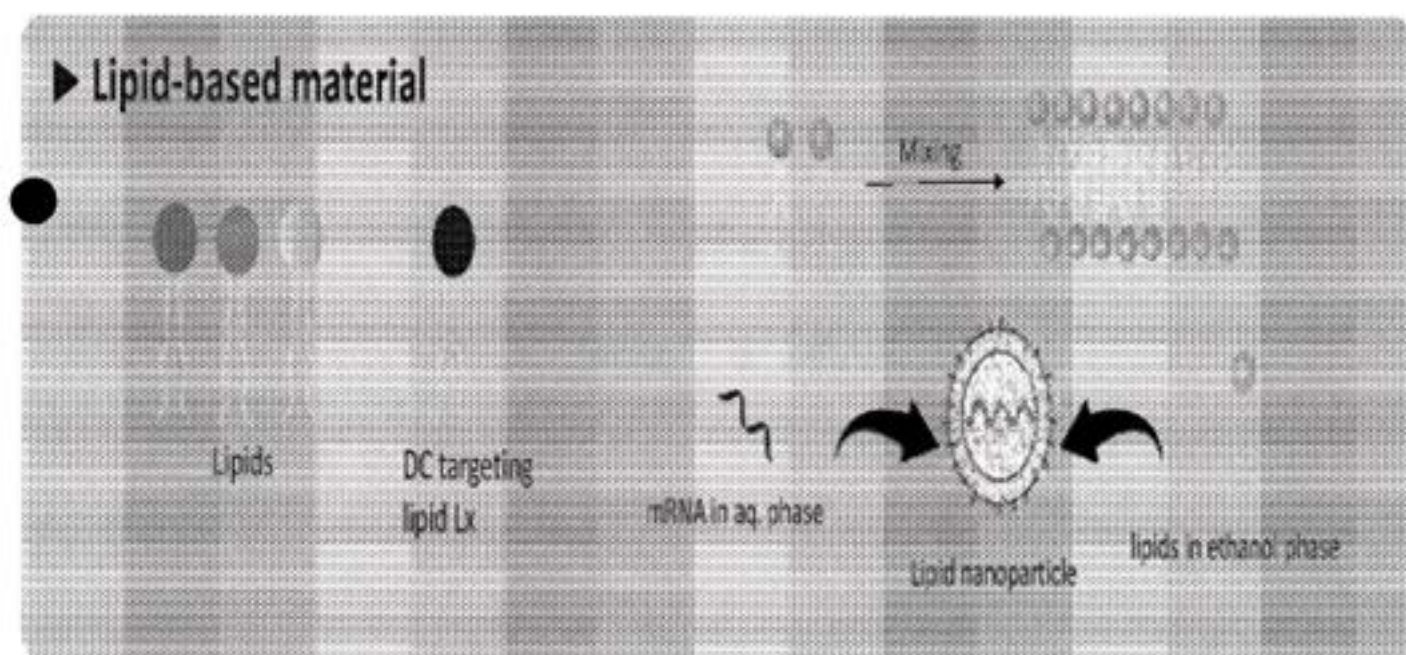
C2C12 muscle cell uptake



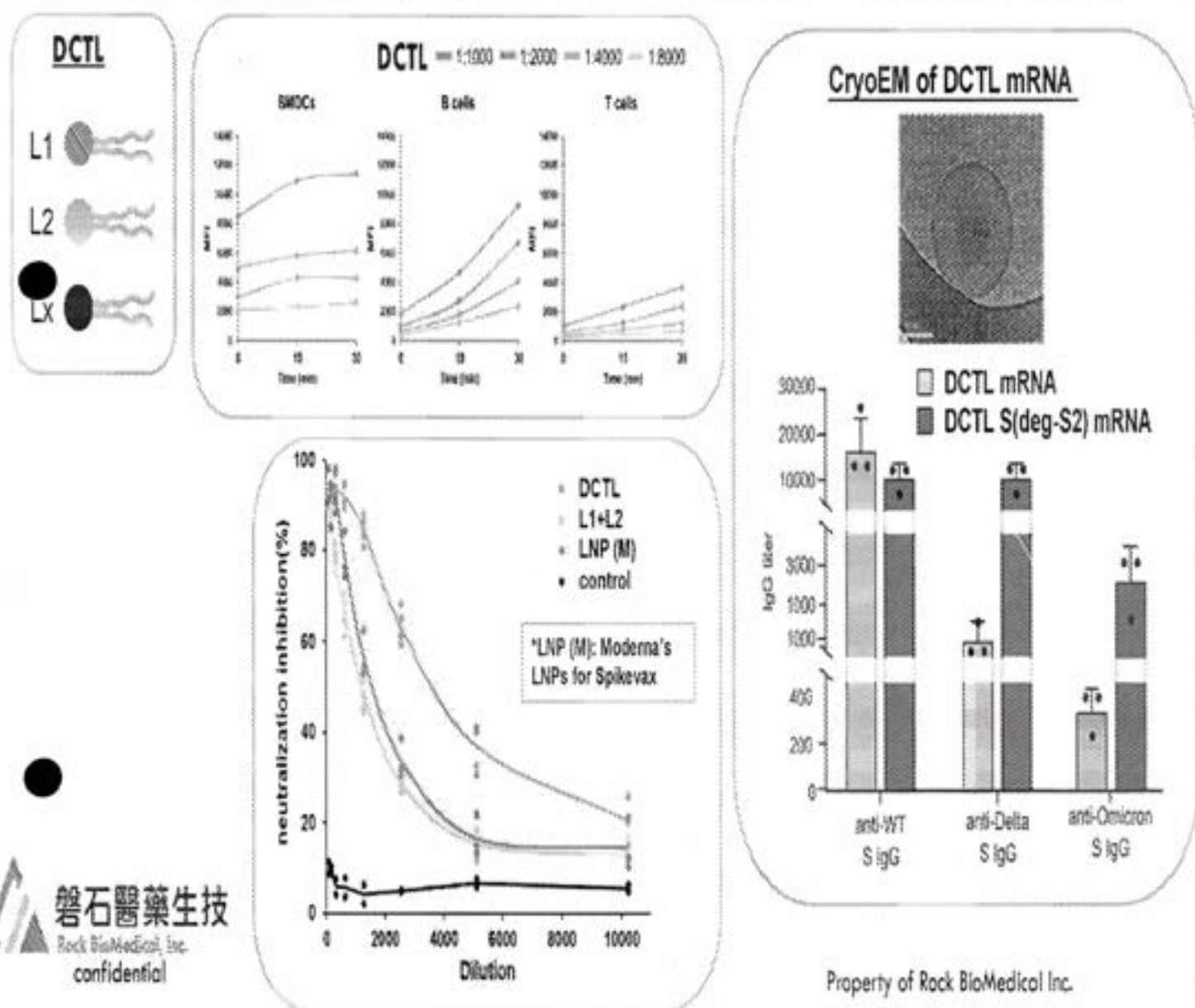
Assessment of Immunogenicity



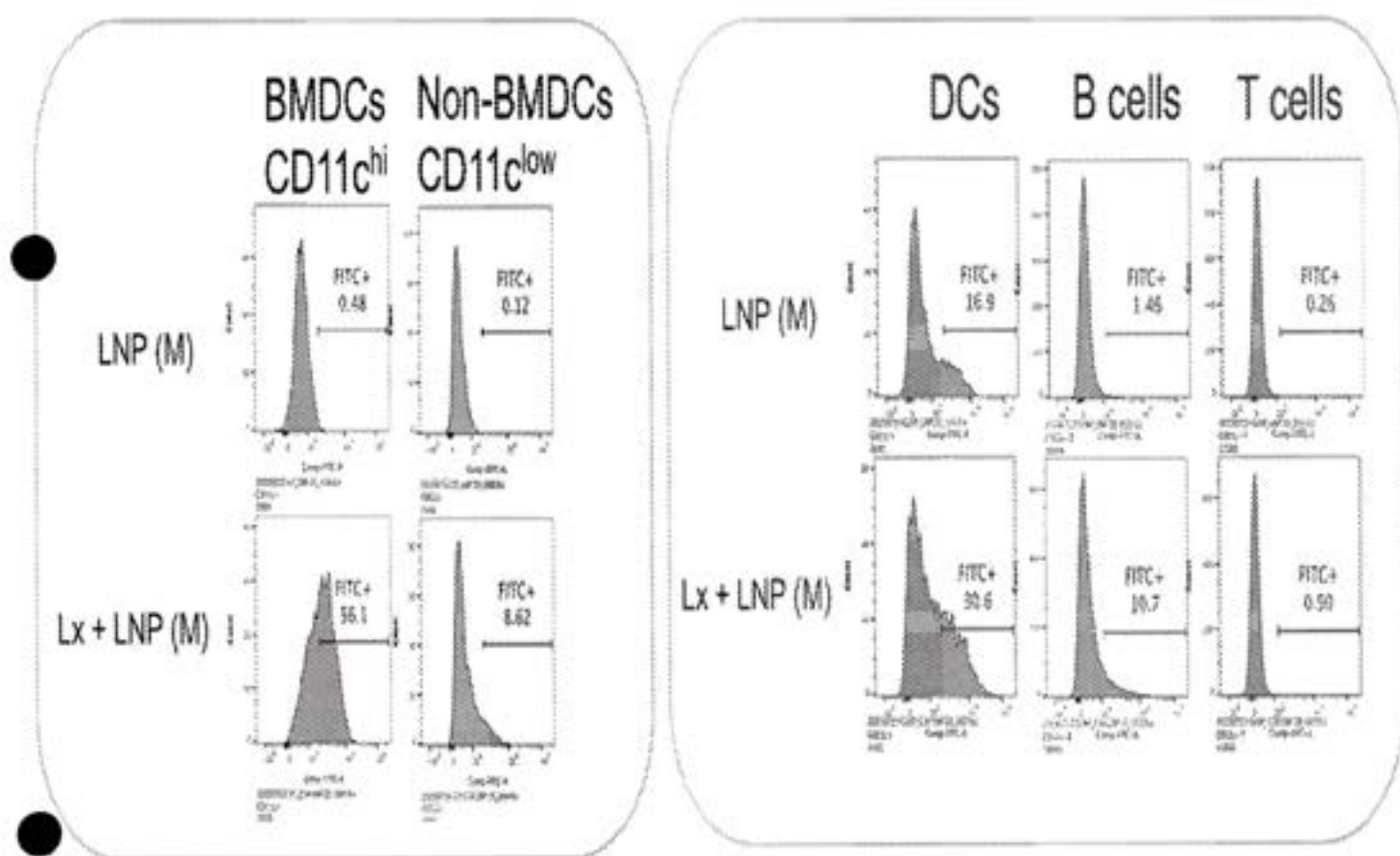
Carrier materials for novel mRNA vaccine formulations



Novel dendritic cell targeting LNPs (DCTL) yields enhanced uptake by DC and improved immune response



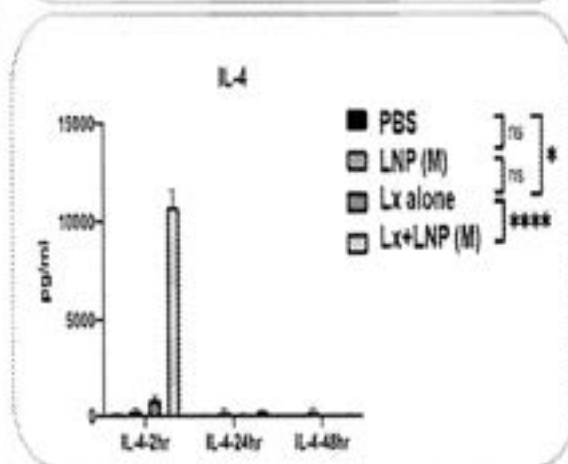
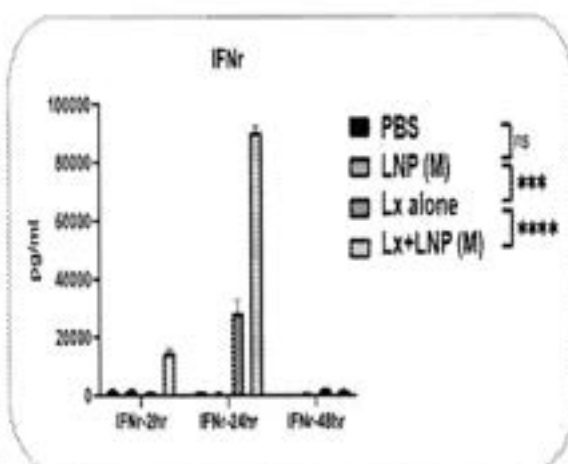
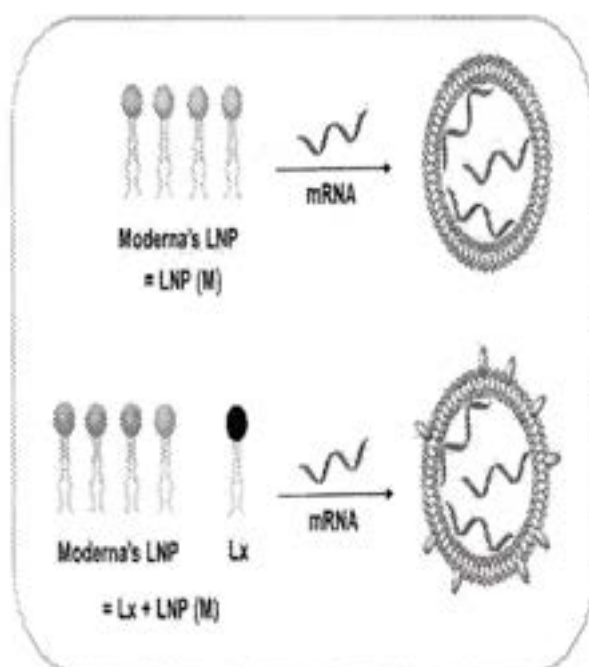
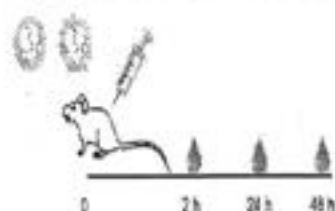
Incorporation of DC targeting lipid Lx improved LNP uptake by BMDCs



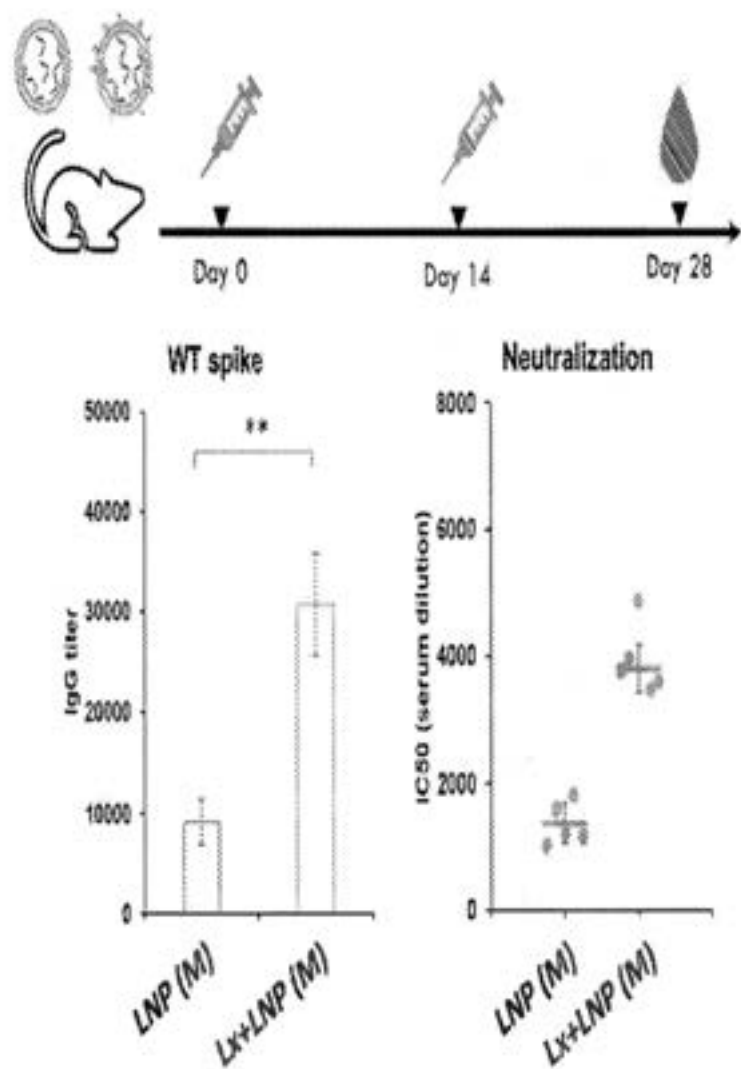
*BMDCs: bone marrow derived dendritic cells; LNP (M): Moderna's LNPs for Spikevax; Lx: DC targeting lipid

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Incorporation of DC targeting lipid Lx improved cytokine responses after immunization



Incorporation of DC targeting lipid Lx improved humoral response after immunization



Summary

- Glycan-protein interaction represents a major way in which glycan structures mediate biological activities. By glycan engineering on both polymer- and lipid-based materials, important breakthrough has been made in our development of novel dendritic cell targeting mRNA vaccine formulations.
- A glycan conjugated lipid Lx effectively enhanced uptake by dendritic cells and improved immune response either as a component of a three-lipid proprietary LNP formulation, or as an additive to the approved LNP formulation used by Moderna's Spikevax.
- The glycan engineering platform can be expanded for mRNA delivery to other cell types for future development.

Thank you for your attention

For collaboration/partnership, please contact:

- Jeng-Shin Lee, MD, PhD

- +886-2-2788-5808 ext 111

- jslee@rockbiomedical.com.tw

- <http://www.rockbiomedical.com.tw>

- Rm. 301, Bldg. A, 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist.,

- Taipei City 115202, Taiwan



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION # 63/587,231	RECEIPT DATE / TIME 10/02/2023 01:25:06 PM ET	ATTORNEY DOCKET # A1000-01300
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Title of Invention

NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS

Application Information

APPLICATION TYPE	Utility - Provisional Application under 35 USC 111(b)	PATENT #	-
CONFIRMATION #	2122	FILED BY	Shannon Warner
PATENT CENTER #	62910280	FILING DATE	-
CUSTOMER #	169053	FIRST NAMED INVENTOR	Chi-Huey Wong
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kay Yang

Documents

TOTAL DOCUMENTS: 4

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01300_20231002_ADS_TBF.pdf	8	Application Data Sheet	2224 KB
A1000-01300_20231002_Spec_TBF.pdf	18	Specification	3423 KB
A1000-01300_20231002_Drawings_TBF.pdf	18	Drawings-other than black and white line drawings	3423 KB
A1000-01300_20231002_Petition_Color_Drawings_TBF.pdf	2	A petition from Applicant to accept Color Drawings or Color Photographs	83 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
A1000-01300_20231002_ADS_TBF.pdf	CFB223F3978F98A49899C4E2391A3B72C9885FAC26A471F7C61E3B7FE92A1B974EAAAC53F21B6DA3C7EFB954EDDC23ED5C9D019FD42B9F316C9CDA1495705858E
A1000-01300_20231002_Spec_TBF.pdf	768695FE1EF4A6B286CAFE80C681DDB8692D16F3D04AD5EEBFA600273120B16E335D4B3BD17C99E5331C5866D781276037E8F476B80C473BEEDE35BC8F64A094
A1000-01300_20231002_Drawings_TBF.pdf	D8CF855CF946D5687589EE60F85F58A743CD654C77E170F1C2BF599D4161822DB3F7B986FF898BD805B40D1AE47D9B390E9FAF5BEF1BFA2B8A835F06FF0041A9
A1000-01300_20231002_Petition_Color_Drawings_TBF.pdf	A9AEE625CCF5864E9E5A959F77638170E5731044CFD7B2513E58A7BF2FB0CFF694734547D1A1F5F4B67AC00A9965DAAE7302B724FB792B85BB147C1401EECC00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01300
		Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chi-Huey		Wong		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Rancho Santa Fe	State/Province		Country of Residence	US
Mailing Address of Inventor:					
Address 1		P.O. Box 8154			
Address 2					
City	Rancho Santa Fe	State/Province			
Postal Code	92067	Country	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jeng Shin		LEE		
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service					
City	Taipei city	Country of Residence	TW		
Mailing Address of Inventor:					
Address 1		c/o Rock BioMedical Inc., Rm 301, Bldg. A, No.99			
Address 2		Ln. 130, Sec. 1, Academia Rd., Nangang Dist.			
City	Taipei City	State/Province			
Postal Code	115202	Country	TW		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Got 581

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01300
		Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Customer Number	169053		
Email Address	ipdocketing@prosylogroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		
Attorney Docket Number	A1000-01300	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	18	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☒ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	169053		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01300
	Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)¹ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ¹	Filing Date (YYYY-MM-DD)	Remove
			Access Code ¹ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01300
		Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01300
	Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

☒ Assignee

☐ Legal Representative under 35 U.S.C. 117

☐ Joint Inventor

☒ Person to whom the inventor is obligated to assign.

☐ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name

Rock BioMedical Inc.

Mailing Address Information For Applicant:

Address 1

Rm 301, Bldg A No. 99, Ln. 130, Sec. 1

Address 2

Academia Rd., Nangang Dist., 115202

City

Taipei City

State/Province

Country

TW

Postal Code

115202

Phone Number

Fax Number

Email Address

Additional Applicant Data may be generated within this form by selecting the Add button.

Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01300
		Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		

Assignee 1				
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
Remove				
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country ⁱ	Postal Code			
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add				

Signature: Remove				
NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).				
This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, all joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of all joint inventor-applicants.				
See 37 CFR 1.4(d) for the manner of making signatures and certifications.				
Signature	/Kay Yang/		Date (YYYY-MM-DD)	2023-10-02
First Name	Kay	Last Name	Yang	Registration Number 61072
Additional Signature may be generated within this form by selecting the Add button. Add				

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PTO/AIA/14 (01-22)

Approved for use through 05/31/2024. OMB 0651-0032

U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01300
		Application Number	
Title of Invention	NOVEL DENDRITIC CELL TARGETING mRNA VACCINE FORMULATIONS		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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**DOCUMENT MADE AVAILABLE UNDER THE
PATENT COOPERATION TREATY (PCT)**

International application number:	PCT/US2024/023597
International filing date:	08 April 2024 (08.04.2024)
Document type:	Certified copy of priority document
Document details:	Country/Office: US
	Number: 63/588,932
	Filing date: 09 October 2023 (09.10.2023)
Date of receipt at the International Bureau:	07 May 2024 (07.05.2024)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

CERTIFICATE OF AVAILABILITY OF A CERTIFIED PATENT DOCUMENT IN A DIGITAL LIBRARY

The International Bureau certifies that a copy of the patent application indicated below has been available to the WIPO Digital Access Service since the date of availability indicated, and that the patent application has been available to the indicated Office(s) as of the date specified following the relevant Office code:

Document details: Country/Office: US

Filing date: 09 Oct 2023 (09.10.2023)

Application number: 63588932

Date of availability of document: 09 Nov 2023 (09.11.2023)

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Date of issue of this certificate: 07 May 2024 (07.05.2024)

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 07, 2024

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 63/588,932
FILING DATE: October 09, 2023

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS US63/588,932



Certified by

Kathi

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
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APPLICATION #
63/588,932

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ATTORNEY DOCKET #
A1000-01400

Title of Invention

Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants

Application Information

APPLICATION TYPE Utility - Provisional Application under
35 USC 111(b)

PATENT # -

CONFIRMATION # 6852

FILED BY Shannon Warner

PATENT CENTER # 62957463

FILING DATE -

CUSTOMER # 169053

FIRST NAMED
INVENTOR Chi-Huey WONG

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Kay Yang

Documents

TOTAL DOCUMENTS: 4

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01400_ADS_20231009.pdf	8	Application Data Sheet	2224 KB
A1000-01400_Figures_20231009.pdf	5	Drawings-other than black and white line drawings	543 KB
A1000-01400_Specification_20231009.pdf	27	-	689 KB
A1000-01400_Specification_20231009-SPEC.pdf	(1-22) 22	Specification	281 KB

A1000-01400_Specification_20231009-DRW.NONBW.pdf	(23-27)	5	Drawings-other than black and white line drawings	538 KB
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Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
A1000-01400_ADS_20231009.pdf	1C225066CD5E576288567633FE454682C56E92346A7DC9076C0EDF6756FC4EDF18C1C8F19631069434B1B07EB706ECAC698CCA8C5CA1E97C5127F1A60884FF8D
A1000-01400_Figures_20231009.pdf	1E0CF4D74732FFF457306FB165B5B62A1C20E7CB29E18AB3825478926E04C527C617C922A54B05EBFD41FDCD042DA7460487BD765881096BAB0A13F3B095932C
A1000-01400_Specification_20231009.pdf	7A4EC9BCF6A3D6B14BBEEC43AC1AF52B560106C83E68D8D89456AB6D6B78F9994D6DC4B804F1840BC34C5AA870B4D9E831CFF1AD822B13AF1F9164FD216FBD14
A1000-01400_Specification_20231009-SPEC.pdf	9CC95115D7F1DFAE8516BB4D558A656B7B9739F407D5EB39EE6EAC1634287F2A06D48FD3C7497515BF578950AFFEC56647171E26758D3B44F196B79570D80615
A1000-01400_Specification_20231009-DRW.NONBW.pdf	E6E1509144DA9B71762A2327EEA500E3B78225C438AB9046B802AAC33F36C2595F4D50618A6E05D73CE2262E2F926C1C3B5426D0823979D11A3710099E1A9E9F

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If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chi-Huey		WONG		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Rancho Santa Fe	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1		PO Box 8154			
Address 2					
City	Rancho Santa Fe	State/Province	CA		
Postal Code	92067	Country	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jeng Shin		LEE		
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service					
City	Taipei City	Country of Residence	TW		
Mailing Address of Inventor:					
Address 1		c/o Rock BioMedical Inc., Rm 301, Bldg. A, No. 99			
Address 2		Ln. 130, Sec. 1, Academia Rd., Nangang Dist.			
City	Taipei City	State/Province			
Postal Code	115202	Country	TW		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					
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Correspondence Information:

6/9
596

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Customer Number	169053		
Email Address	ipdocketing@prosytagroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		
Attorney Docket Number	A1000-01400	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☒ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	169053		

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78. When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

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This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)¹ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country ¹	Filing Date (YYYY-MM-DD)	Access Code ¹ (if applicable)
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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐	This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

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B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

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Sdy

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01400
	Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1			Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
Clear			
☒ Assignee	☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor	
☒ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor:			
If the Applicant is an Organization check here. ☒			
Organization Name	Rock BioMedical Inc.		
Mailing Address Information For Applicant:			
Address 1	Rm 301, Bldg A No. 99, Ln. 130, Sec. 1		
Address 2	Academia Rd., Nangang Dist., 115202		
City	Taipei City	State/Province	
Country	TW	Postal Code	115202
Phone Number		Fax Number	
Email Address			
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

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Address 1			
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See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Kay Yang/		Date (YYYY-MM-DD)	2023-10-09	
First Name	Kay	Last Name	Yang	Registration Number	61072

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01400
	Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PNAS

Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants

Chi-Huey Wong

Jeng Shin Lee

Abstract

Since the outbreak of SARS-CoV-2 in 2019, more than 15 million spike protein sequences have been identified, raising a new challenge for development of a broadly protective vaccine against the various emerging variants. We found that the virus, like most other human viruses, depends on host-made glycans to shield the conserved epitopes on spike protein from immune response and demonstrated that deletion of the glycan shields exposed highly conserved epitopes and elicited broadly protective immune responses. In this study, we identified 17 conserved epitopes from 14 million spike protein sequences and 11 of the conserved epitopes are in the S2 domain, including the 6 most conserved epitopes in the stem region. We also demonstrated that deletion of the glycosites in the spike mRNA S2 domain or the stem region exposed the highly conserved epitopes and elicited broadly protective immune responses,

particularly CD-8⁺ T cell response against various SARS-CoV-2 variants, and other human coronaviruses including MERS and SARS viruses.

Significance Statement

In an effort toward development of a universal vaccine targeting the highly conserved epitopes on SARS-CoV-2 and variants, we examined 14 million sequences of spike proteins reported by Global Initiative on Sharing All Influenza Data (GISAID) and identified 17 linearly conserved epitopes, with 11 located in the S2 domain including the 6 most conserved epitopes in the stem region. Deletion of the glycosites in the S2 domain or the stem region exposed the glycan-shielded conserved epitopes and elicited broadly protective immune responses against SARS-CoV-2 variants and other human coronaviruses, including MERS and SARS viruses.

Introduction

Since the outbreak of Severe Acute Respiratory Syndrome Virus-2 (SARS-CoV-2) in 2019 in Wuhan, China, the virus has spread rapidly around the world and continued to change with mutation. As of February 15, 2023, the virus and its variants infected more than 650 million people and caused more than 6.6 million deaths (1, 2). A great deal of global efforts has been directed toward development of effective means to contain the pandemic, and among them, vaccination has been the most effective. Of the many types of vaccine candidates available, the successful development of mRNA vaccines represents a breakthrough in the field (3). After administration of an mRNA vaccine, it is translated *in vivo* to the real protein antigen to elicit immune responses, so

there is no need to manufacture the protein vaccine *in vitro*. However, mRNA vaccines are unstable and must be modified and formulated with lipid nanoparticles (LNPs) for storage and administration (3). Currently, all mRNA vaccines are developed based on the surface spike protein of the virus as an immunogen. However, various emerging variants, such as Omicron subvariants, are able to significantly escape the immune responses to the spike protein (4). Therefore, to develop a broadly protective vaccine against the current and upcoming variants, it is necessary to analyze the huge number of SARS-CoV-2 S protein sequences in the GISAID (Global Initiative on Sharing All Influenza Data) database (5, 6) and identify the conserved sequences as targets for development of vaccines with broad protection and long-acting immunity.

Our previous study showed that human viruses are all coated with host-made sugars to facilitate infection and to shield the conserved epitopes from immune response, and deletion of the glycan shields from spike protein exposed highly conserved epitopes and elicited broadly protective immune responses (7). In addition, the SARS-CoV-2 mRNA vaccine with deletion of glycosites in the S2 domain of the spike protein elicited broadly protective antibody and memory CD8⁺ T-cell responses against the highly conserved epitopes shielded by glycans (8, 9). This low-sugar vaccine approach, conceived in 2009 for universal influenza vaccine development (10), was also demonstrated as an effective strategy for development of a broadly protective HIV vaccine (11), and the approach is also consistent with the recent report that the spine helix in S2 (the stem) is highly conserved and can be a possible epitope for universal vaccine design (12). To continue this line of study, we identified 17 conserved

epitopes from more than 14 million S protein sequences reported to GISAID, including those with less than 1% residue mutation rates. Of these 17 conserved epitopes, 11 are in the S2 domain, including 6 in the CD/HR2 (stem) region. In this and previous analyses of the spike protein sequences, we found that the 6 linear epitopes in the stem are the most conserved but shielded by the glycans from the 6 *N*-glycosites in this region. The other conserved epitopes are spread in different domains. Based on this finding, we believe that deletion of the glycans in the stem or the S2 domain will expose the highly conserved epitopes and elicit broadly protective immune responses. In this study, we showed that the spike mRNA vaccine from the Wuhan strain or the Delta strain with deletion of the 9 glycosites in the S2 or the 6 glycosites in the stem region elicited more broadly protective immune responses as compared to the unmodified mRNA vaccine. To further understand the origin of the broad protection, we found that glycosylation affects protein stability and cellular immune response, and in addition to the variants and subvariants of SARS-CoV-2, the glycosite-deleted mRNA vaccine is capable of eliciting cross-reactive immune responses against not only SARS-CoV-2 variants but also alpha and beta coronaviruses including MERS and SARS viruses.

Results

Seventeen linearly conserved epitopes are identified in the S protein of SARS-CoV-2. Previously, we identified 12 conserved epitopes from 6 million S protein sequences, including those with residue mutation rates less than 0.1%; 7 of the 12 conserved epitopes are in the receptor binding domain (RBD) and 5 in the S2 domain (8). However, 4 of the 7 conserved epitopes in the RBD have mutations in Omicron

subvariants, thus reducing the effectiveness of vaccines and therapeutic antibodies that target the RBD domain (13). In this study, we used a less than 1% mutation rate to identify 17 conserved epitopes from 14 million S protein sequences.

Simulated 3D structures of S proteins, with and without the 66 N-glycans and 6 O-glycans were obtained based on our previous research (7). By calculating the relative solvent accessibility (RSA) of each amino acid of the spike protein and comparing the two types of structures, three states of each residue designated as exposed, shielded by glycans, and buried, can be identified. Mutation information provided by the GISAID database was then used to identify which amino acids are conserved and not affected by mutations in dominant strains, including the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, BA.2.12.1, BA.2.75.*), and top-ranked emerging variants (e.g., XBB.1.5, XBB.1.16, XBB.1.9.1, BQ.1.1, EG.5 etc.) reported by GISAID. The above information was integrated to confirm that the sequences identified are conserved with continuous amino acid sequences (10-20 in length). The IEDB (The Immune Epitope Database) (14) was also used to confirm that these sequences were epitopes (please check Materials and Methods for more details).

Fig. 1 shows the seventeen linearly conserved epitopes identified in this study. One of them (E1) is in the NTD, three (E2-E4) in RBD, two (E5-E6) in SD1/2, and eleven (E7-E17) in the S2 domain, including 6 in the CD/HR2 (the stem) region, which are the most conserved epitopes in this analysis. Except for the E17, all conserved epitopes were shielded by glycans, suggesting that during the antigen presentation process, the glycans may not be completely processed and thus may shield the conserved epitopes

from immune cell recognition. In the figure, single asterisks indicate the eight conserved epitopes with less than 0.5% mutation rate of each amino acid in the epitopes and double asterisks indicate the nine highly conserved epitopes with less than 0.1% mutation rate of each residue in the epitopes. Interestingly, 6 of these highly conserved epitopes are concentrated in the stem (E12-E17) and 3 in the RBD (E2-E4). It is also noted that E3 is in the receptor-binding motif (RBM, 438-506) of RBD, the site for S protein binding to ACE2. The conservation of E3 suggests that it is probably essential for pathogen-host interaction.

We then divided all residues of S protein into three categories for analysis: buried, shielded, and exposed residues (the residue number in each category is 851:172:250). The average mutation rates of the three categories were 0.84%, 1.67%, and 3.66%, and the standard deviations were 5.90%, 8.33%, and 11.58%, respectively. Buried residues are those not easily recognized by the immune system and have the lowest mutation rate, followed by shielded residues and the highest exposed residues. Based on this analysis, the conserved S protein epitopes are concentrated in the stem region of S2 as shown in Fig. 2. Thus, deleting the 9 glycosites in S2 or the 6 glycosites in the stem to generate the low-sugar spike protein or its mRNA as vaccine is expected to better expose the conserved and glycan-shielded epitopes to the immune system and thus elicit broadly protecting immune responses against the conserved epitopes.

SARS-CoV-2 and SARS-CoV share the same epitopes in the stem. We were curious whether these conserved epitope sequences in the S protein of SARS-CoV-2 were also found in the S protein of SARS-CoV. Therefore, we performed a pairwise sequence

alignment between the S protein sequences of SARS-CoV-2 and SARS-CoV. The result (Fig. S1) showed that 5 of the 17 conserved SARS-CoV-2 epitopes are identical to SARS-CoV (E13-E17), and interestingly, they are all located in the stem region (CD and HR2). Among the 6 conserved epitopes (E12-E17) in the stem of the SARS-CoV-2 S protein, 5 epitopes (E12-E16) are shielded by glycans. Moreover, of these 6 epitopes (E12-E17), E14 has residue mutation rates of less than 0.5%, while the other four epitopes have residue mutation rates of less than 0.1%. This indicates that the conservation of the stem is relatively high, making it a suitable target for development of broadly protective vaccines. We also examined the E12 epitope located in the CD region of SARS-CoV-2 S protein and found that the sequence differed from SARS-CoV by only one residue (DVVIGI[V/I] NNTVY). Thus, we concluded that the vaccine with deletion of S2 or stem glycosites might also be effective against SARS-CoV, and this indeed was supported by the finding in our previous study of mono-GlcNAc decorated SARS-CoV-2 S protein (7).

The spike mRNA vaccine with deletion of glycosites in S2 increases the breadth of antibody neutralization. Since the spike mRNA vaccine from the Wuhan (WH) strain with deletion of the 9 glycosites in S2 provided a broad protection against SARS-CoV-2 variants (8), we used Wuhan (WH) and Delta variants (15) as models for comparative study. To study how the mRNA vaccine of S protein with deletion of glycosites in S2 affected S protein expression and immune response, we deleted the *N*-glycosites (from N to Q in the N-X-S/T sequon) where the conserved epitopes were shielded by glycans. After confirming the expression of the prefusion S protein in the

HEK293 cell line (Fig. 3A), the mRNA of WH strain S protein with deletion of all *N*- and *O*-glycosites (deg-S) did not express the S protein well. The mRNA that encoded the S protein, the S protein with deletion of S2 glycosites, or the S protein with deletion of all glycosites was then encapsulated in lipid nanoparticles (LNPs) to form mRNA-LNP for immunization in mice. Compared to the unmodified spike mRNA vaccine, sera from mice immunized by the spike mRNA with deletion of all S2 *N*-glycosites in WH S (WH S-(deg-S2)) elicited a slightly lower (50 -67%) IgG titer against the fully glycosylated WH S (Fig. 3B) or deglycosylated WH S protein (Fig. 3C) but a higher (7 fold) IgG titer against the fully glycosylated Delta S (Fig. 3D) or deglycosylated Delta S protein in enzyme-linked immunosorbent assay (ELISA) (Fig. 3E). Similarly, the Delta spike mRNA vaccine with deletion of glycosites in S2 (Delta S-(deg-S2)) elicited a slightly lower IgG titer against the fully glycosylated Delta S protein (Fig. 3D) but a higher IgG titer against the fully glycosylated or deglycosylated WH S (Fig. 3B, C) and the deglycosylated Delta S protein (Fig. 3E). A similar result was observed in the case of spike mRNA vaccine with deletion of the 6 glycosites in the stem region (WH S-(deg-CD/HR2)) (Fig. 3F-H) (16). The mice immunized with WH S-(deg-CD/HR2) mRNA vaccine induced a stronger antibody response (10 fold) with a higher IgG titer against the S protein of Delta (Fig. 3G) and Omicron BA.1 (Fig. 3H) variants as compared to the unmodified mRNA, suggesting that deleting the glycan shield in the stem would enhance antibody response to the stem with cross-reactivity against the S protein of other variants containing the conserved epitopes.

To analyze the effect of S2 glycosite deletion on the neutralization activity of antibodies generated from immunized mice, the pseudovirus neutralization assay was performed, and the result showed that the WH and Delta mRNA vaccines with deletion of glycosites in S2 generated antibodies with slightly reduced 10 % of neutralization activity against WH or Delta pseudovirus, respectively (Fig. 4A, B), but with increased 5-8 fold of neutralization activity against the Omicron variants including BA.1 (Fig. 4C), BA.1.12.1 (Fig. 4D), BA.2 (Fig. 4E), BA.4 and BA.5 (Fig. 4F), than the unmodified mRNA. This finding was consistent with our previous study that WH mRNA vaccine with deletion of all S2 N-glycosites induced a broader protection against the alpha, beta, gamma and delta strains, perhaps due to the conserved antibody response to the conserved epitopes after deletion of the shielded glycans (8).

Glycosylation affects protein stability and cellular immune response. To

characterize the T cell response, splenocytes from immunized mice were isolated and incubated with the peptide pool of WH S, RBD, and S2 protein to measure granzyme B (GrzB)-secreting T cells by enzyme-linked immune absorbent spot (ELISpot) analysis. It was found that the mRNA vaccine with deletion of S2 glycosites induced more GrzB-secreting cells than the unmodified spike mRNA of WH and Delta (Fig. S2A-C). The Delta S-(deg-S2) with more stable S protein expression than that of the WH S-(deg-S2) showed a weaker T cell response (Fig. S3). Interestingly, the WH S-(deg-S) vaccine with all glycosites deleted showed the lowest stable protein expression but the highest T cell response. These results indicate that unlike the case *in vitro*, the stability and

integrity of the S protein generated *in vivo* affects T cell response and the misfolded S protein seemed to induce a stronger T cell response.

The S2 glycosite-deleted mRNA vaccine elicited cross-reactive immune response against alpha and beta coronaviruses, as well as MERS and SARS viruses. To investigate whether the antibodies induced by the spike mRNA vaccine with deletion of S2 glycosites provide a protection against human alpha and beta coronaviruses since the S2 domain and the stem region contain more conserved epitopes than the other domains among alpha and beta coronaviruses, SARS-CoV-2 variants and MERS as well as SARS virus (12, 17), it was shown that sera from mice immunized with the S2-glycosite deleted spike mRNA had higher (2-3 fold) IgG titers against human alpha (HCoV-NL63 (Fig. S4A) and HCoV-229E (Fig. S4B)) and beta (HCoV-HKU1 (Fig. S4C), HCoV-OC43 (Fig. S4D), MERS-CoV (Fig. S4E) and SARS-CoV (Fig. S4F)) coronaviruses, and increased 6-8 fold of neutralization activity against MERS-CoV (Fig. 4G) and SARS-CoV (Fig. 4H) in pseudovirus neutralization assay, suggesting that the spike mRNA vaccine with deletion of glycosylation on S2 provides broad protection against pan-coronaviruses.

Discussion

We have identified 17 conserved epitopes in the SARS-CoV-2 S protein, and 11 of which (more than 60%) are in the S2 region including 6 in the stem region, and 5 of the 6 conserved stem epitopes are also conserved in the stem of SARS-CoV, MERS-CoV and human alpha and beta coronaviruses. In our previous analysis of 4 million S protein sequences, seven conserved epitopes were found in the RBD; however, four of these

seven conserved epitopes, including 331-346, 368-378, 441-450, and 458-470 have mutations in the Omicron variants. In contrast, the 11 conserved epitopes in the S2 domain, especially the 6 in the stem, are still highly conserved and are more reliable as targets for vaccine development. We analyzed the mutations in the S protein O-glycosites and found that the mutation rates of T323 and S325 were relatively low, only 0.09% and 0.01%, respectively. Regarding the *N*-glycosites, we investigated if mutation of any of the 22 *N*-glycosite sequons (N-X-S/T) affected *N*-glycosylation and found that the T19R of Delta and T19I of Omicron mutations (BA.2, BA.2.12.1, BA.2.75.*, BA.4, BA.5, BQ.1.1, XBB.1.5, XBB.1.16, etc.) changed the sequons from NLT to NLR and from NLT to NLI respectively and consequently caused a loss of *N*-glycosylation at the nearby *N*-glycosite N17. Other sequons, including those in S2 and stem, were not affected significantly, so deleting the glycans from S2 or stem exposed the conserved epitopes and elicited broadly protective immune responses. In addition, recent studies have shown that the fusion inhibitors of the pan-coronavirus are also effective against SARS-CoV-2 (18), and human antibodies recognizing the fusion peptide and stem provide a broad protection against coronaviruses (12, 17, 19). These observations are consistent with our finding that the S2 domain or the stem of coronavirus is an excellent target for vaccine development.

Repeated immunization with homologous antigens, such as in the case of influenza A vaccine, may increase antibody responses to the conserved epitopes, albeit with glycan shield and provide cross-reactivity against heterologous strains (20). People receiving the third dose of the SARS-CoV-2 mRNA vaccine (BNT162b2 manufactured

by Pfizer-BioNTech and mRNA-1273 manufactured by Moderna based on the WH strain) showed protection against variants such as Omicron variants at least transiently, and cross-reactivity against heterologous strains with increased antibody titers (21). Interestingly, repeated SARS-CoV-2 WH S vaccination also elicited neutralizing antibodies targeting the stem region (12, 17, 22). However, immunization with spike mRNA vaccine with deletion of S2 glycosites elicited a stronger antibody and T cell response against pan-coronaviruses, suggesting that the induced immune responses target the conserved epitopes in the S2 region. In addition, the WH spike mRNA vaccine with deletion of the 6 glycosites in the stem also induced an antibody response with increased IgG titer against the S protein of Delta and Omicron variants, suggesting that the SARS-CoV-2 spike mRNA vaccine with deletion of stem glycosylation elicits antibodies that recognized the conserved epitopes in the stem (Fig. S2). Under the selective pressure from vaccines, glycosylation on the conserved epitopes of S protein may play an essential role in maintaining the proper tertiary and quaternary structures and simultaneously shielding the conserved epitopes from immune response. The highly conserved epitopes mainly located in the stem region of SARS-CoV-2 are also highly conserved among coronaviruses of the four genera. Therefore, neutralizing antibodies targeting the conserved epitopes in the stem should provide cross-reactive protection against pan-coronavirus (7, 8, 15, 16, 22). On the other hand, deletion of the glycan shields in the highly conserved stem domain or the S2 domain to generate low-sugar vaccines should better expose the highly conserved epitopes and elicit enhanced and broadly protective immune responses.

The serum from the mice immunized with the SARS-CoV-2 S vaccine was overall similar to human convalescent serum (23), and that from SARS-CoV-2 S2 DNA-vaccinated mice reacted strongly with an epitope in the HR2 and membrane-proximal region that was highly conserved among SARS-CoV-2 variants (7, 24). However, using the recombinant WH S2 protein without glycosylation produced by *Escherichia coli* failed to induce neutralizing antibodies against SARS-CoV-2 WH or Omicron S pseudovirus, perhaps due to the conformation change *in vitro* (24). However, the SARS-CoV-2 spike mRNA vaccines from the WH and Delta S mRNA with deletion of glycosites in S2 or stem elicited enhanced neutralizing antibody and CD8⁺ T-cell responses against SARS-CoV-2 variants and other coronaviruses, suggesting that the misfolded S protein generated *in vivo* from the mRNA with deletion of glycan shields can still be processed to elicit immune responses.

T-cell response prevented SARS-CoV-2 infection from progressing to severe conditions (25–27). In addition, CD8⁺ T cell response induced by prior infection provided approx. 80%-95% protection against SARS-CoV-2 variant reinfections for more than 8 months (9, 28, 29). In this study, we have demonstrated that the SARS-CoV-2 spike mRNA with deletion of glycosites in the S2 or stem domain reduced the stability of S protein, thereby triggering a strong memory CD8⁺ T cell induction.

Human viruses, including influenza A virus (IAV) and SARS-CoV-2 use host-made glycans to facilitate infection and to shield the conserved epitopes on the virus surface from immune response. Vaccination with mono-glycosylated hemagglutinin (HA) or inactivated IAV induced cross-strain protection against influenza virus infections (10, 30-

31). Interestingly, the HA stem-specific antibodies are broadly protective against most type A influenza viruses (32, 33), and previous studies showed that deletion of the glycans in HA elicited a high level of stem-specific antibodies (34-37) and T cell response (37). Based on the study of influenza and SARS-CoV-2, this low-sugar vaccination strategy is expected to be a general and effective approach to development of universal vaccines against other human viruses.

Materials and Methods

Conserved Epitope Identification. The 3D structural models of the SARS-CoV-2 S protein with representative glycan profiles were obtained in our previous work (7). The probe radius of 7.2Å, mimicking the hypervariable loops in the complementarity determining region of antibodies, was used in the FreeSASA program (38) to calculate each RSA of residue in S protein, both with and without representative glycans. The classification of exposed/buried residues was consistent with the definition in the previous study by Kajander *et al.* (39). Glycans are considered to provide shielding for the residues with buried states in models with glycans whereas these same residues have exposed states in models without glycans. A total of 14,624,495 SARS-CoV-2 S protein sequences and their variant information were extracted from the GISAID database (version: March 3, 2023) for amino acid mutation rate calculation. The linear conserved epitopes in this study are 10-20 contiguous amino acids in length and the state of the residues are mostly exposed or exposed but shielded by glycans. All amino acid mutation rates in the conserved epitope sequences should be <1% and not

affected by mutations in the dominant virus strains and the top-ranked emerging variants. The dominant virus strains were defined as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (BA.1, BA.2, BA.3, BA.4, BA.5, BA.2.12.1, BA.2.75.*). The top-ranked emerging variants (spread) reported by GISAID are BA.2.47, BQ.1, BQ.1.1, BQ.1.1.28, BQ.1.1.32, CH.1.1.3, EG.5.1, EL.1, EU.1.1, FD.1.1, XBB.1.16, XBB.1.16.6, XBB.1.17.1, XBB.1.5, XBB.1.5.10, XBB.1.5.59, XBB.1.9.1, XBB.1.9.2, XBB.2.3, XBB.2.3.3, XBB.2.3.8, and XBF. (version: February 8, 2023, April 24, 2023, June 13, 2023, and August 19, 2023). All variants are double-confirmed by GISAID and CoV-SPECTRUM (40). All conserved sequences are confirmed by IEDB as epitopes with 100% concordance or as their subsequences.

Cell lines. The Human embryonic kidney cells (HEK293) and Huh7 human hepatoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Rockville, MD) with 10% heat-inactivated fetal bovine serum (FBS) (Thermo Scientific) and antibiotics (100 U/ml penicillin G and 100 gm/ml streptomycin).

Antibodies and proteins. The rabbit anti-SARS-CoV-2 S polyclonal antibody was purchased from ABclonal. SARS-CoV-2 full-length WH and Delta S proteins (293T cell expressed) were purchased from Royez. HCoV-NL63, HCoV-229E, HCoV-HKU1, and MERS-CoV spike protein were purchased from Sino Biologicals (Beijing, China). HCoV-OC43 spike protein was obtained from Acrobiosystems. SARS-CoV spike protein was purchased from Biotechne. Mouse monoclonal anti-GAPDH was obtained from Millipore. To obtain the deglycosylated S protein, WH or Delta S proteins were deglycosylated in a buffer solution with PNGase F (Sigma) at 37°C for 24 h in the dark. After deglycosylation, samples were purified and checked by Western blot (7, 34).

mRNA vaccine of deglycosylated S protein and formulation. The pre-fusion state of the S, the codon-optimized S gene of SARS-CoV-2 was synthesized by GenScript and cloned into pVax or pMRNATM, and was stabilized by proline substitutions at positions K968 and V969 (S-2P) (41). To delete the *N*-glycosites, the putative sequon N-X-S/T was changed to Q-X-S/T by using site-directed mutagenesis on the S-2P expression plasmid. For the in-vitro transcription, the linear DNA with the T7 promoter, 5' untranslated region, 3' untranslated region, S-2P, and poly(A) tail signal sequence was amplified by using TOOLS Ultra High Fidelity DNA Polymerase (BIOTOOLS Co., Ltd., Taipei, Taiwan) with 1 µl of the DNA template in an mMESSAGE mMACHINE® Kit (Thermo Scientific) at 37°C for 1hr according to the manufacturer's protocol. The mRNA was purified by an RNA cleanup kit (BioLabs), according to the manufacturer's protocol, and stored at -80°C until further use. For the formulation mRNA-LNP, mRNA was encapsulated in LNP using a self-assembly process in which an aqueous solution of mRNA at pH 4.0 was rapidly mixed with an ethanolic lipid mixture containing ionizable cationic lipid, phosphatidylcholine, cholesterol, and polyethylene glycol-lipid (8, 42). The compositions of LNP were DSPC (Sigma), cholesterol (Sigma), DOTAP (Sigma), and DMG-PEG 2000 (Sigma). The mRNA-LNP was characterized and subsequently stored at -80°C at a concentration of 1 mg/ml. After HEK293 cells were transfected with 10 µg of mRNA-LNP in six wells of a plate at 48 hrs, the total cell lysate was collected to monitor the expression of S by western blot (8).

Animals and immunizations. BALB/c mice aged 6-8 weeks old (*n* = 5) were immunized intramuscularly with 50 µg mRNA-LNP in PBS with 300 mM sucrose.

Animals were immunized at week 0, boosted with a second vaccination at week 2, and

serum samples and spleens were collected from each mouse one week after the booster immunization. The animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee of Academia Sinica (8).

Serum IgG titer measure. Anti-S protein ELISA was used to determine IgG titer. Plates were coated with 50 ng/well of variant S protein as shown in Fig. 3 and S3, and then blocked with 5% skim milk. The serum from immunized mice and HRP-conjugated secondary antibodies were sequentially added. Peroxidase substrate solution (TMB) and 1M H₂SO₄ stop solution were used and absorbance (OD 450 nm) was read by a microplate reader (8).

Pseudovirus neutralization assay for serum study. SARS-CoV-2 pseudovirus variants were constructed by the RNAi Core Facility at Academia Sinica using the procedure described previously (8). The pseudotyped lentivirus was then stored at -80°C. To estimate the lentiviral titer by AlarmaBlue assay (Thermo Scientific), the transduction unit (TU) of pseudotyped lentivirus was estimated by using a cell viability assay. HEK-293T cells expressing the human ACE2 gene were plated on a 96-well plate one day before lentivirus transduction. To determine the titer of pseudotyped lentivirus, different amounts of lentivirus were added into the culture medium containing polybrene (final concentration 8 µg/ml) (Sigma), and spin infection was carried out at 1,100 xg in 96-well plate for 30 min at 37°C. After incubation for 16 h, the culture medium containing virus and polybrene was removed and replaced with fresh complete DMEM containing 2.5 µg/ml puromycin (Sigma). After treating puromycin for 48 h, the culture medium was removed, and the cell viability was detected by using AlarmaBlue reagents according to the manufacturer's instructions. The survival rate of uninfected

cells was set as 100%, and the virus titer was determined by plotting the survival cells versus the diluted viral dose (8). The SARS-CoV and MERS-CoV pseudoviruses were purchased from eEnzyme.

For neutralization assay, heat-inactivated sera or antibodies were serially diluted with desired dilution 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 or Huh7 (for MERS-CoV pseudovirus) 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 post-infection and continuously cultured for another 48 h. The expression level of the luciferase gene was determined by using 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 and the calculation formula as shown below: $(RLU_{control} - RLU_{Serum}) / RLU_{control}$ (7, 8).

Measurement of GrzB-secreting cells. A total of 5×10^5 splenocytes from immunized mice were *ex vivo* restimulated with full-length WH S, RBD, and S2 peptide mix (0.1 µg/ml final concentration per peptide) (Sino Biologicals) in the GrzB ELISpot assays (R&D Systems) according to the manufacturer's instructions and spots were counted (8).

DNA plasmid transfection and MG132 treatment. After the HEK293 cell was seeded in the 6 well plates, cells were transfected with 3 µg of each plasmid by TransIT®-LT1 Transfection Reagent (Mirus) and then incubated with 1 µM MG-132

(MedChemExpress) or DMSO at 37°C for 24 h. The total lysate was collected and the variant S expression was analyzed by western blot (8).

Statistics and reproducibility. All data were presented as means $\pm$ standard error of the mean. The numbers of samples and replicates of experiments were shown as mentioned in the figure legends. Comparisons between groups were determined using the student's t-test. Differences were considered significant at * $P < 0.001$, ** $P < 0.05$. All data were analyzed using GraphPad Prism 6 software.

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41. R. N. Kirchdoerfer, *et al.*, Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Sci. Rep.* **8**, 15701 (2018).
42. N. Pardi, *et al.*, Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J. Controlled Release* **217**, 345–351 (2015).

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Figures

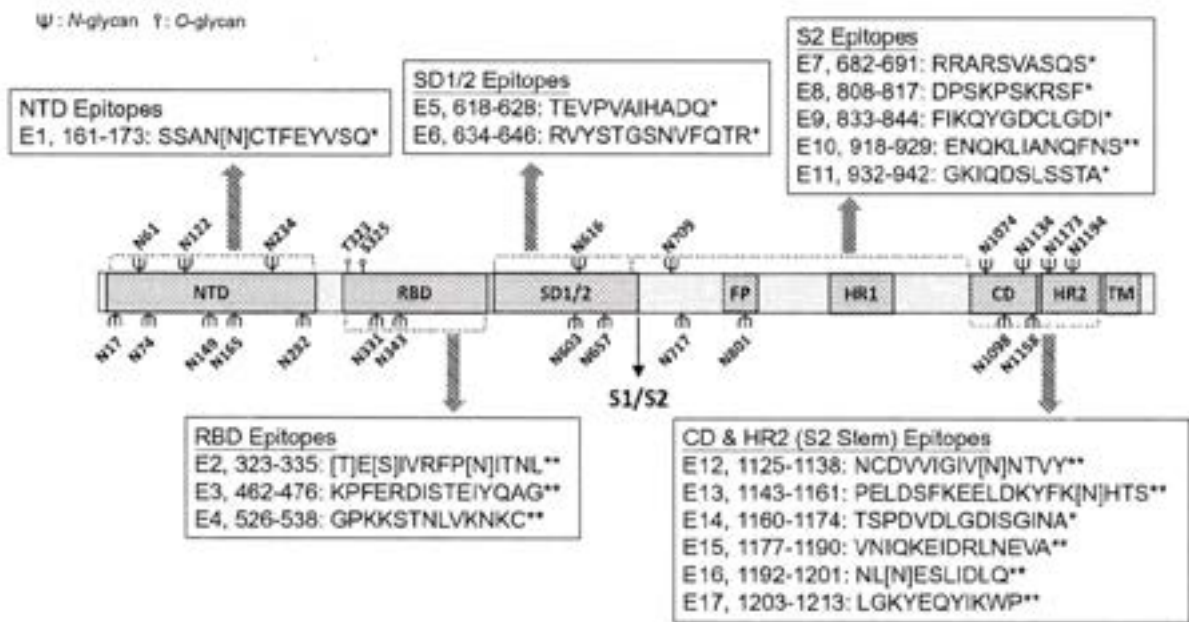


Figure 1. We have identified seventeen linear, conserved surface epitopes (E1-E17) on the SARS-CoV-2 spike glycoprotein with less than 1% residue mutation rates. All epitopes except E17 are shielded by glycans. The 11 conserved epitopes located in the S2 region have residue mutation rates of either less than 0.5% (indicated by a single asterisk) or less than 0.1% (indicated by a double asterisk, most concentrated in the stem region of the S2 domain). Removal of glycans from the S2 domain or the stem region can expose these epitopes and is useful for universal vaccine design. Note that residues in square brackets represent either *N*- or *O*-glycosites.

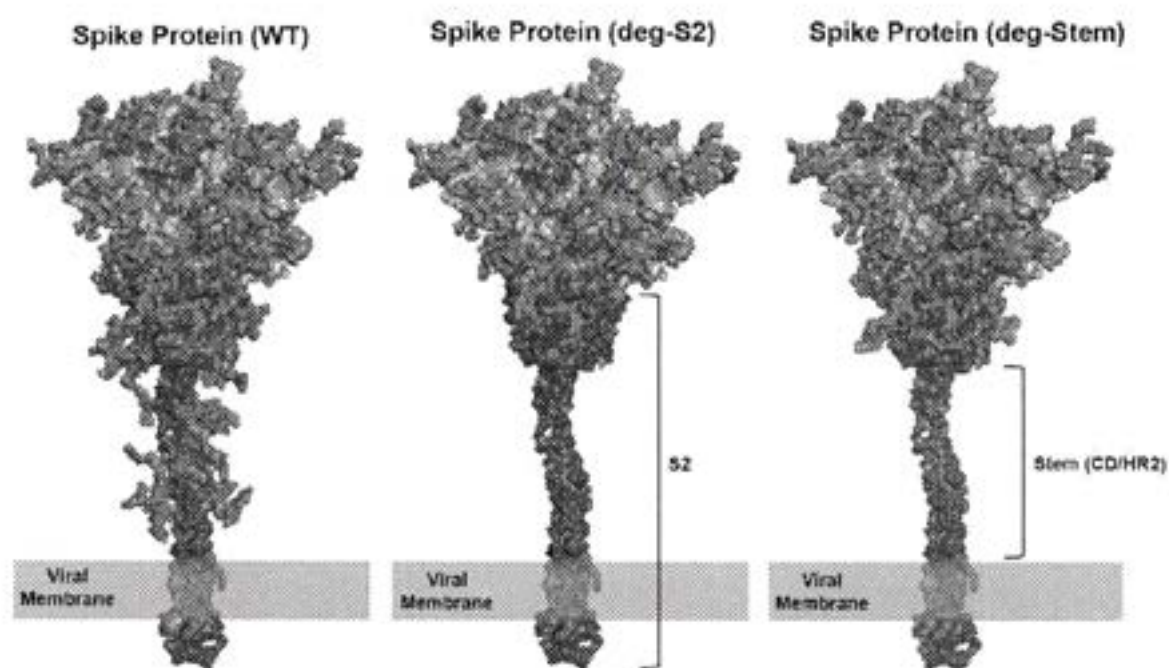


Figure 2. The 3D wild type (WT), deg-S2 (deletion of 9 glycosites), and deg-Stem (deletion of 6 glycosites) spike glycoprotein structures of SARS-CoV-2 are labeled with conserved surface regions and glycans. Gray: S1 conserved region. Dark gray: S2 conserved region. Blue: Glycans. Red: Regions are not suitable for broadly vaccine development, including mutated residues in dominant strains, residues with mutation rates of more than 1%, and buried residues. Orange: transmembrane domain.

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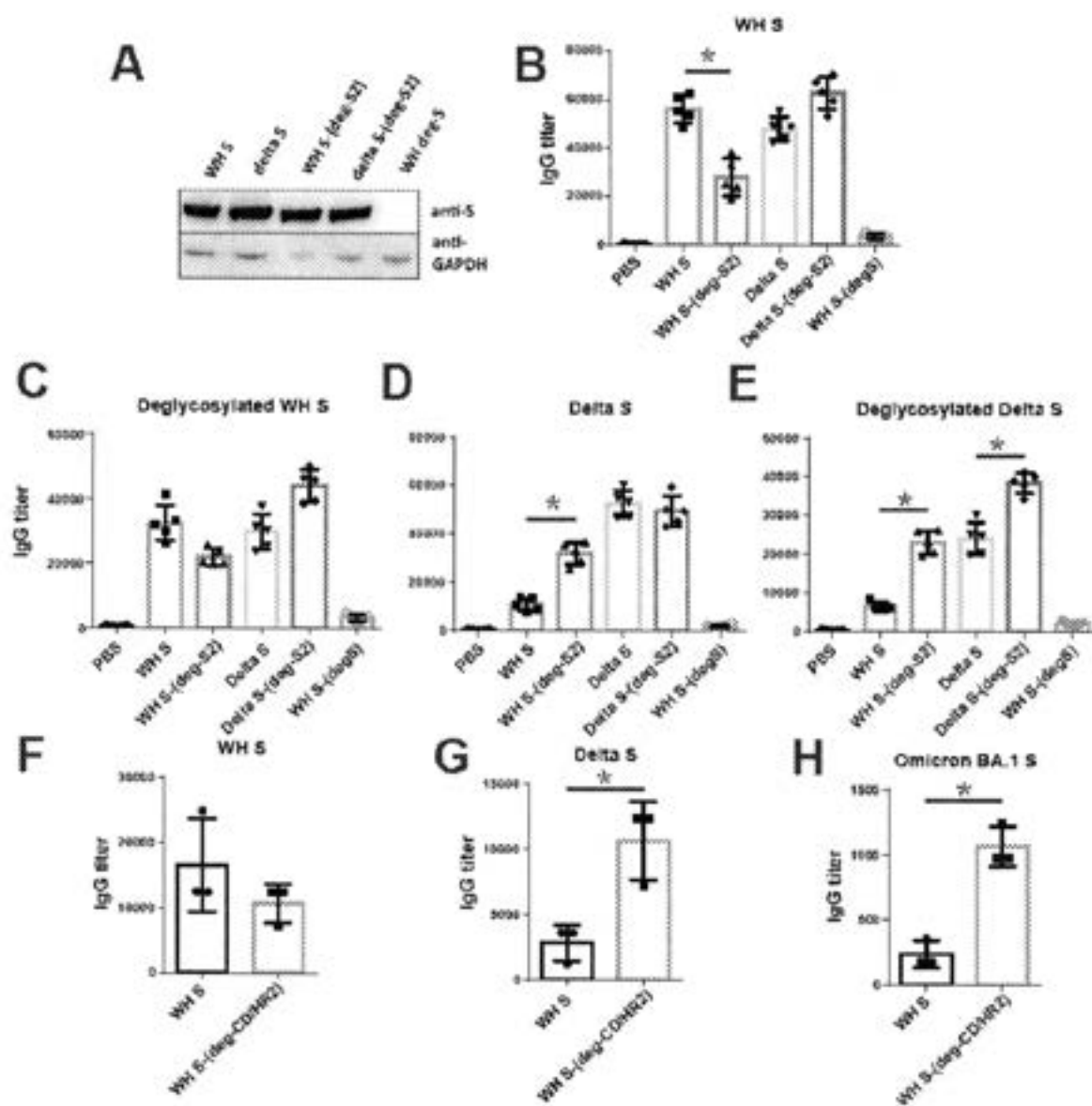


Figure 3. Effect of spike glycosylation on antibody production and specificity. (A), Analysis of S protein expression after transfection with mRNA at 48 h by Western blot. The filter was probed with anti-S and anti-GAPDH monoclonal antibodies. Humoral immune response in BALB/c mice was shown as a protein-specific IgG titer from serum against (B) WH S, (C) deglycosylated WH S, (D) Delta S, and deglycosylated Delta S

analyzed by ELISA. The protein-specific IgG titer from the mice that immunized with deglycosylated CD/HR2 S mRNA vaccine. The IgG titer against (F) WH S, (G) Delta S and (H) Omicron BA.1 S were analyzed by ELISA. B-E shows mean $\pm$ SD for five independent experiments. F-H shows mean $\pm$ SD for three independent experiments. *P < 0.001.

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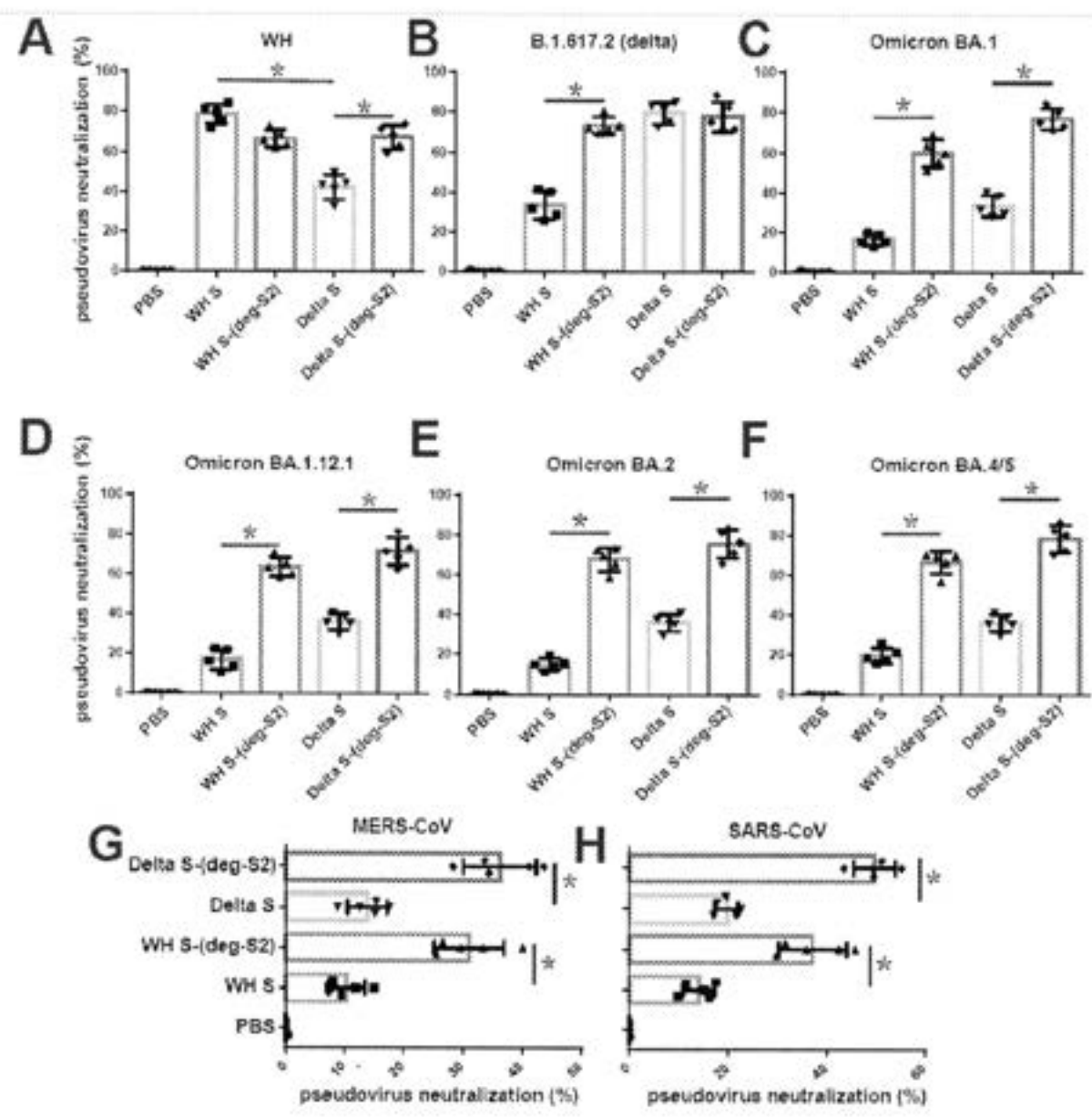


Figure 4. The breadth of mRNA vaccine protection against pseudovirus variants of coronavirus. Neutralization assay of pseudovirus variants are shown with (A) WH, (B) Delta, (C) Omicron BA.1, (D) Omicron BA.1.12.1, (E) Omicron BA.2, (F) Omicron BA.4/5. (G) MERS-CoV and (H) SARS-CoV. (A–H) show mean $\pm$ SD for five independent experiments. * $P < 0.001$.

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Supporting Information for

Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants

This PDF file includes:

Figures S1 to S4



Fig. S1. Pairwise sequence alignment of S proteins shows that five conserved epitopes in CD and HR2 regions between SARS-CoV-2 and SARS-CoV are the same (E13-E17).

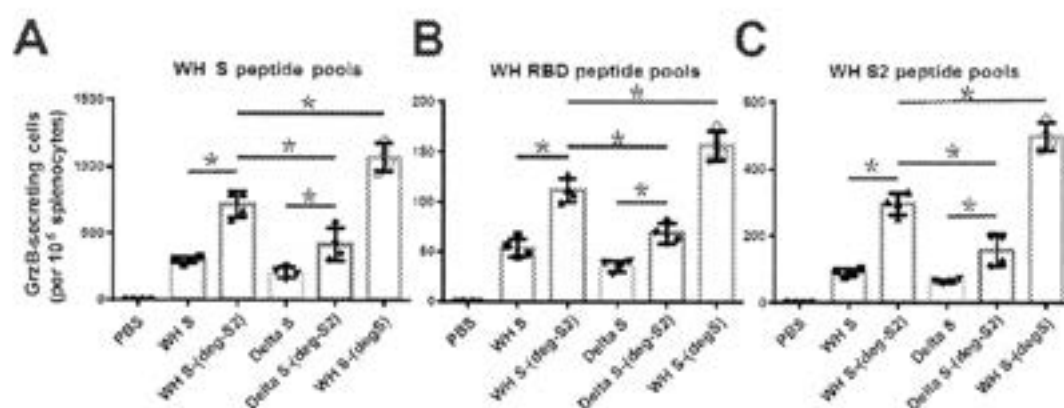


Fig. S2. Glycosylation on S2 affected CD8⁺ T cell response. After incubation of the splenocytes isolated from immunized mice with full-length (A) WH S, (B) WH RBD, and (C) WH S2 peptide pools, the GrzB-secreting cells were measured by ELISpot. (A–C) show mean $\pm$ SD for five independent experiments. * $P < 0.001$.

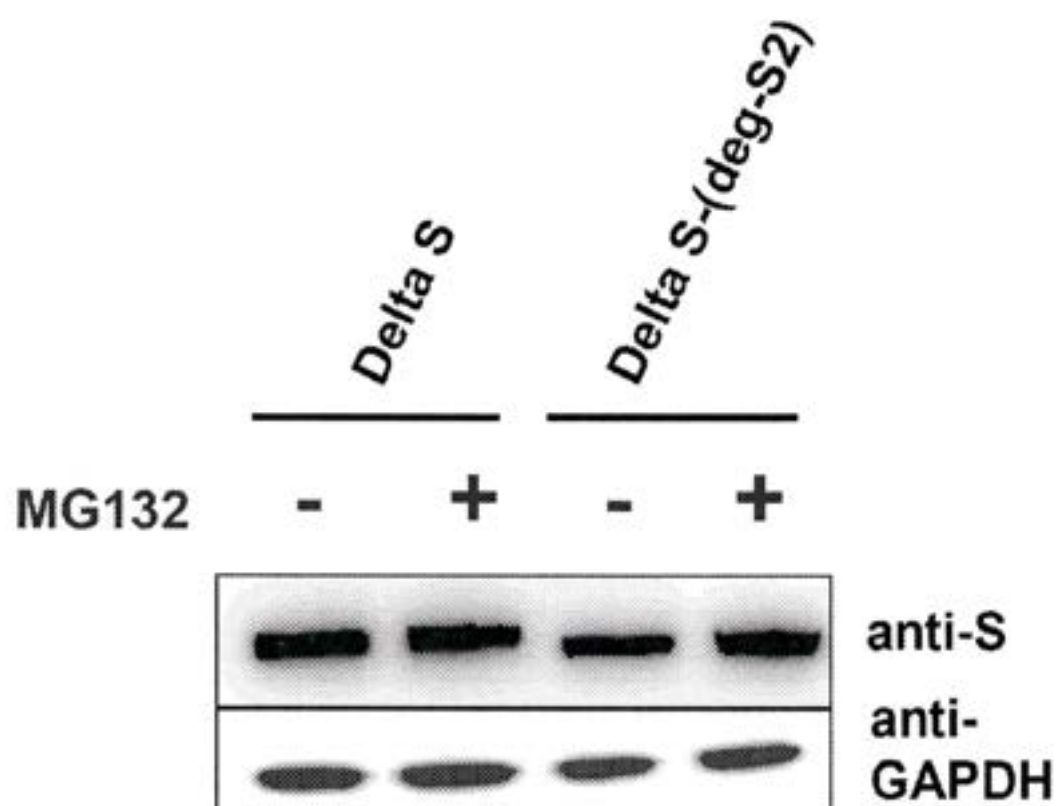


Fig. S3. Analysis of Delta S protein expression in HEK293T cells transfected with plasmids and MG132 treatment by Western blot. The filter was probed with anti-S and anti-GAPDH monoclonal antibodies.

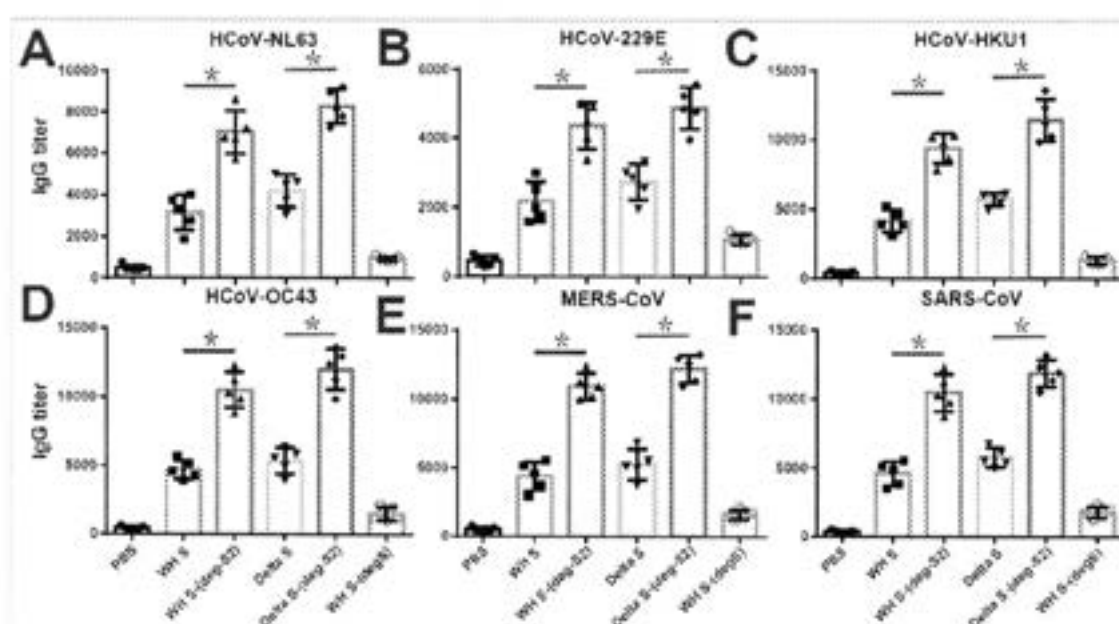


Fig. S4. Characterization of the broadly neutralizing antibody induced by glycosite-deleted S2 mRNA vaccine. Humoral immune response in BALB/c mice was shown as protein-specific IgG titer from serum against (A) HCoV-NL63, (B) HCoV-229E, (C) HCoV-HKU1, (D) HCoV-OC43, (E) MERS-CoV and (F) SARS-CoV analyzed by ELISA. (A–F) show mean $\pm$ SD for five independent experiments. *P < 0.001.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION # 63/588,932	RECEIPT DATE / TIME 10/09/2023 06:46:18 PM ET	ATTORNEY DOCKET # A1000-01400
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Title of Invention

Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants

Application Information

APPLICATION TYPE	Utility - Provisional Application under 35 USC 111(b)	PATENT #	-
CONFIRMATION #	6852	FILED BY	Shannon Warner
PATENT CENTER #	62957748	FILING DATE	-
CUSTOMER #	169053	FIRST NAMED INVENTOR	Chi-Huey WONG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Kay Yang

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01400_SuppADS2_20231009.pdf	8	Application Data Sheet	161 KB
Warning: This is not a USPTO supplied ADS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.			
A1000-01400_Transmittal_Change-Applicant_20231009.pdf	1	Miscellaneous Incoming Letter	90 KB
A1000-01400_Request to Change Applicant_20231009.pdf	2	Request under 37CFR1.46(c) to correct, update, change applicant	296 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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A1000-01400_Request to Change Applicant_20231009.pdf	A4017395A5926EF95C65572095D55AF79E56825F4A7F2B5DA2EF460E14B5475010A3690CC91ED342A4ECB65FF6AC00CB62BCEB9D25E3F9D317D2098C3484A54F

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Supplemental Application Data Sheet

PTO/AIA/14 (01-22)
Approved for use through 05/31/2024. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
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Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chi-Huey		WONG		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Rancho Santa Fe	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1		PO Box 8154			
Address 2					
City	Rancho Santa Fe	State/Province	CA		
Postal Code	92067	Country i	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Jeng Shin		LEE		
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service					
City	Taipei City	Country of Residence i	TW		
Mailing Address of Inventor:					
Address 1		c/o Rock BioMedical Inc., Rm 301, Bldg. A, No. 99			
Address 2		Ln. 130, Sec. 1, Academia Rd., Nangang Dist.			
City	Taipei City	State/Province			
Postal Code	115202	Country i	TW		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					
					Add

Correspondence Information:

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ An Address is being provided for the correspondence information of this application.

Customer Number	169053		
Email Address	ipdocketing@prosyigroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		
Attorney Docket Number	A1000-01400	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☒ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	169053		

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

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This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)¹ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ¹	Filing Date (YYYY-MM-DD)	Access Code ¹ (if applicable)	Remove
Additional Foreign Priority Data may be generated within this form by selecting the Add button.				

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. ☐ NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

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A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

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☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01400
	Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

☒ Assignee	☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor
☒ Person to whom the inventor is obligated to assign	☐ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here: ☒

Organization Name: Rock BioMedical Inc.

Mailing Address Information For Applicant:

Address 1	Rm 301, Bldg A, No. 99, Ln. 130, Sec. 1		
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City	Taipei City	State/Province	
Country	TW	Postal Code	115202
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01400
	Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants	

Assignee 1				
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
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This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, all joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of all joint inventor-applicants. See 37 CFR 1.4(d) for the manner of making signatures and certifications.					
Signature	/Kay Yang/			Date (YYYY-MM-DD)	2023-10-09
First Name	Kay	Last Name	Yang	Registration Number	61072
Additional Signature may be generated within this form by selecting the Add button.					

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01400
		Application Number	
Title of Invention	Low-Sugar SARS-CoV-2 Spike mRNA Vaccines with Deletion of Glycan Shields in the S2 or Stem Domain Elicit Broadly Protective Immune Responses against Coronavirus Variants		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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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 entireties of the aforementioned applications are incorporated herein by reference.

FIELD

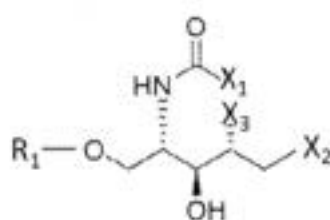
[0002] The instant disclosure relates to novel formulations for targeted nano-delivery. Specifically, the disclosure relates to compositions and methods directed to novel bi-functional nanoparticle formulations capable of selectively delivering a payload to a desired region in a tissue or a specific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

BACKGROUND OF THE INVENTION

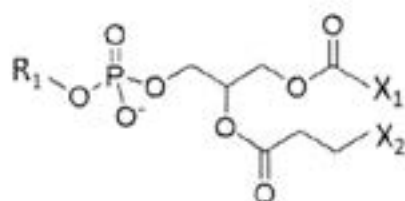
[0003] Localized delivery via nanotechnology has been widely used in scientific, industrial, and clinical applications. It has increasingly become a promising way for drug delivery, providing advantages including improving solubility and penetration of drug molecules. In recent examples of the mRNA vaccines 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 ^1H 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 IFN γ (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

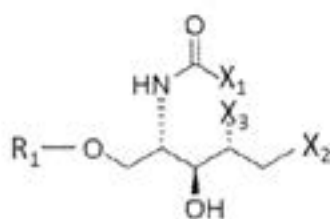
[0025] Nanoparticles have been widely used in various applications. Among them, **lipid nanoparticles (e.g., liposomes)** are the most well-established delivery systems for medicines due to their biocompatibility and biodegradability. A typical liposome has a bilayer structure formed by phospholipids 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.

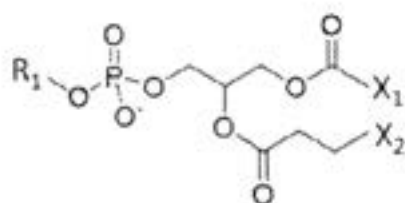
[0027] **TARGETING LIPID COMPOUND**

[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] *R₁ group*

[0031] *Targeting Functionality.* In certain embodiments, the R₁ 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 R₁ 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 R₁ group is designed to have a targeting moiety, which can be a ligand of a receptor on a target cell. For example, the R₁ 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 R₁ 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 R₁ group, which might provide better chances to interact with a dendritic cell.

[0033] In some other embodiments, the R₁ group is configured to target Siglec-1, so the glycosyl group can comprise 9-N-(4H-thieno[3,2-c]chromene-2-carbamoyl)-Neu5Ac- α 2,3-Gal-GlcNAc. In some embodiments, the R₁ group is configured to target Siglec-2, and the glycosyl group can comprise 9-Biphenyl Neu5Ac- α 2,6-Gal-GlcNAc. In some embodiments, the R₁ group is configured to target Siglec-5/E, and the glycosyl group can comprise Neu5Ac- α 2,3-Gal-GlcNAc.

[0034] In some embodiments, the R₁ group comprises a formula of R₂-R_A-, wherein R₂ 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 C₁₋₆ alkyl, halide, or C₁₋₆ 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, R₂ 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 R₁ and a target is Ca²⁺-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 CF₃). In some embodiments, the aryl group is coupled with the targeting moiety. For example, the R₁ group can comprise an O-aryl mannoside.

[0036] *Spacer.* In some embodiments, the attachment group of R₁ comprises a spacer. The spacer is configured to provide structural flexibility to R₁. Without wishing to be bound by theories, the flexibility allows the glycosyl group of R₁ 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 (OCH₂CH₂) 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 (OCH₂CH₂) 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 R₁ group is a substituted glycosyl group. The glycosyl group might comprise 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ 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, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof. For example, the substituted benzene ring can be a phenol group.

[0042] In some embodiments, the R₁ group is a mono-mannoside substituted with 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof. In certain embodiments, the R₁ 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 C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, and sulfite.

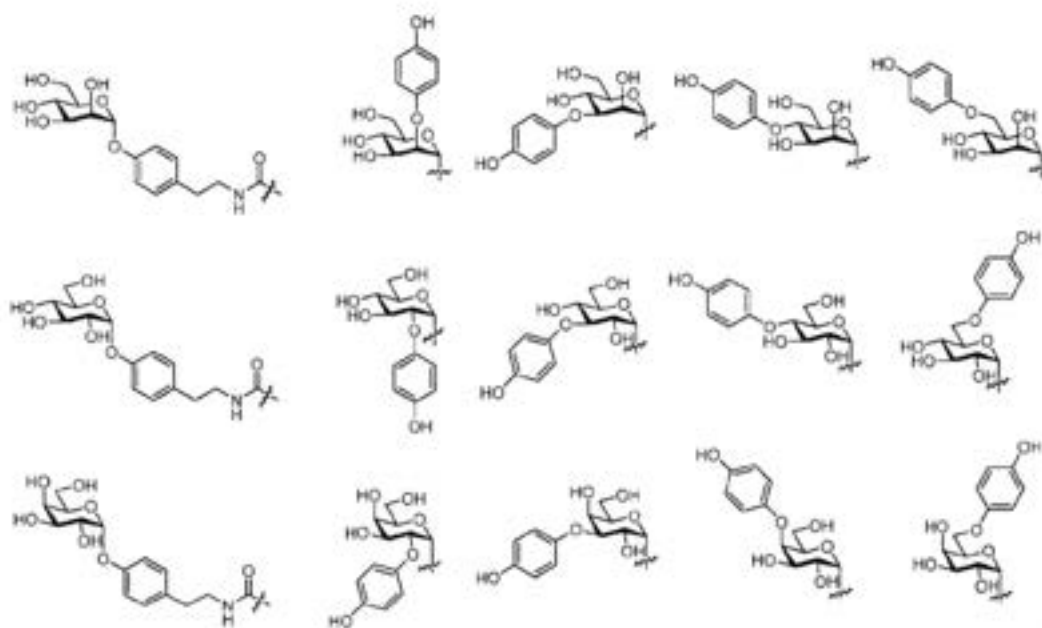
[0043] In some embodiments, the R₁ group comprises a first mannoside and a second mannoside. Each of the first mannoside and the second mannoside is independently substituted with 1 to 6 substituents, and each substituent can be C₁₋₆ alkyl, C₁₋₆ alkenyl, halogen, C₁₋₆ alkyl halide, amine, C₁₋₆ alkyl amine, amide, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

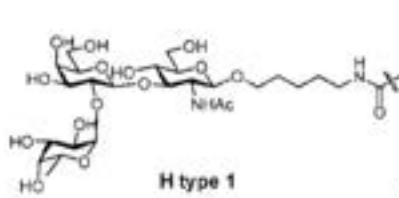
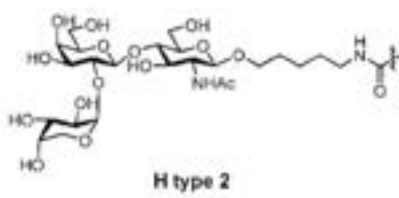
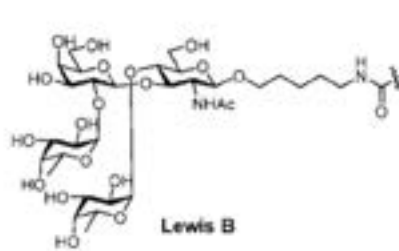
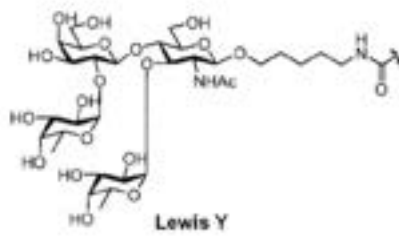
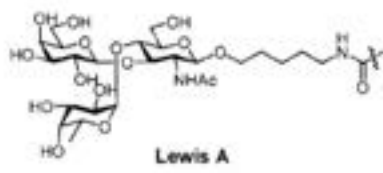
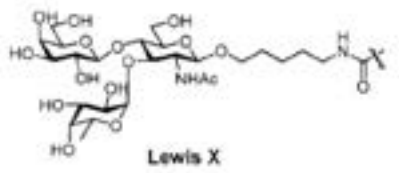
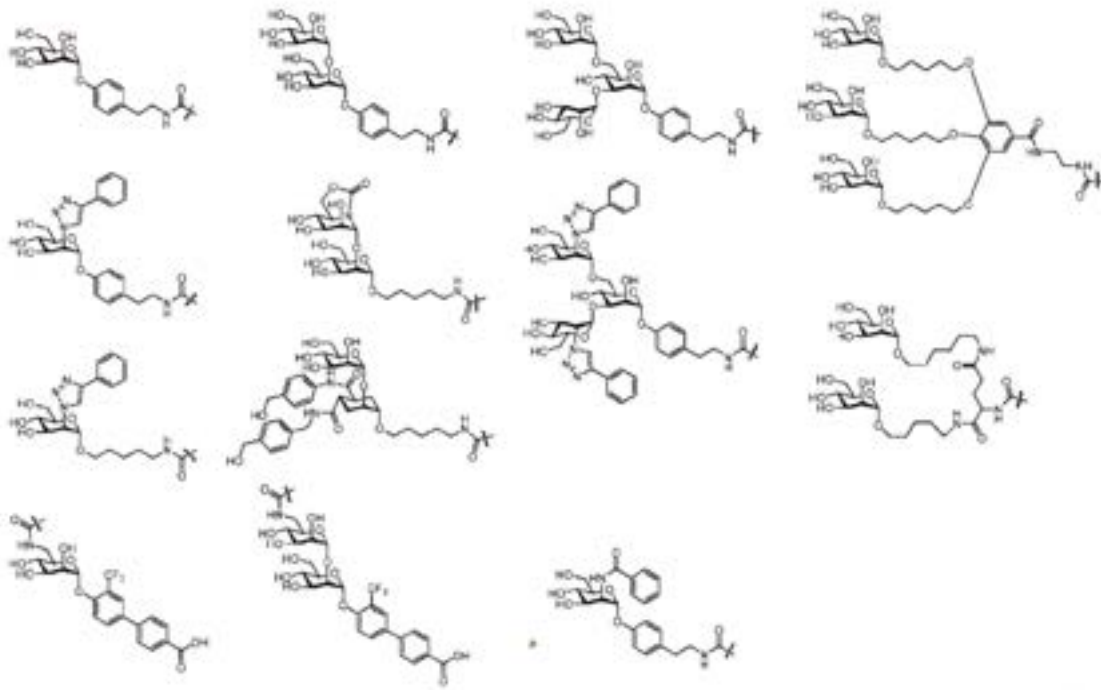
[0044] *Binding affinity.* In some embodiments, the binding affinity between the glycosyl group of R₁ and a target can be defined by a dissociation constant (K_D). In some embodiments, the K_D at pH 7.4 can be 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 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.

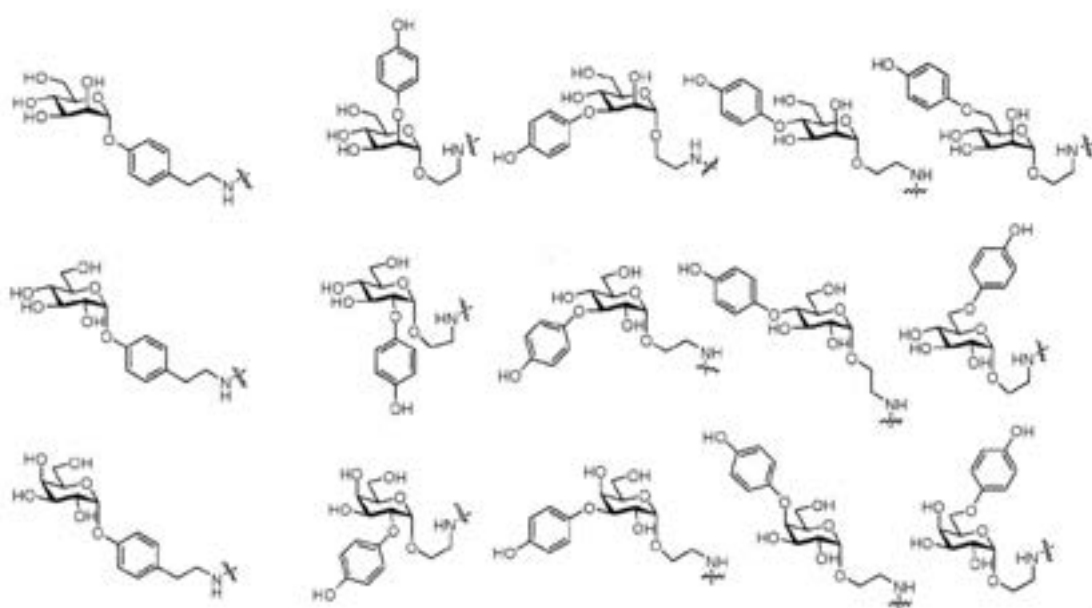
[0045] In some other embodiments, the K_D at pH 5 can be 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1250, 1500, 1750, or 2000 nM, or any range defined by the foregoing endpoints, such as, 1 to 2000, 1 to 1500,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

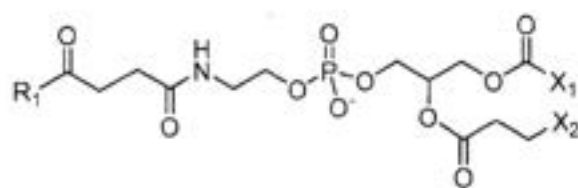




; and

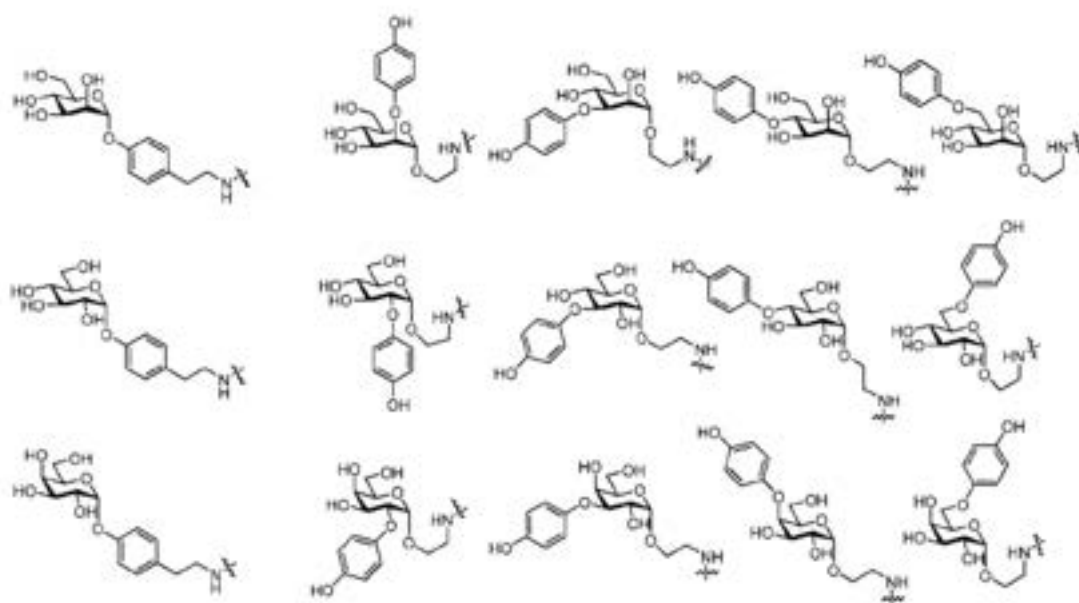


[0047] In some embodiments, the compound of the present disclosure has the structure shown in Formula 3:



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.

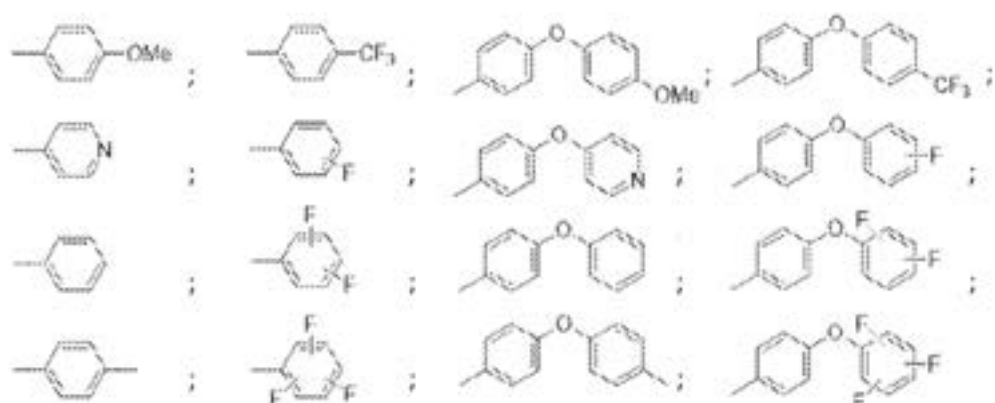
[0052] In some embodiments, X_1 and X_2 are each independently hydrogen, C_{8-30} alkyl, C_{8-30} alkenyl, C_{8-30} alkynyl, aryl, aryloxy, or a substituted version thereof, or $-(CH_2)_nX_4$, n is 8 to 30, and X_4 is hydrogen, aryl, aryloxy, heterocyclic group, or a substituted version thereof, provided 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 .

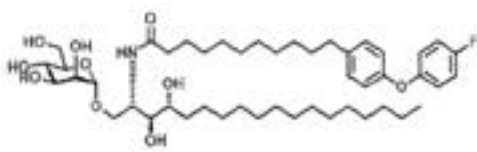
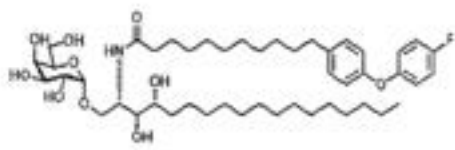
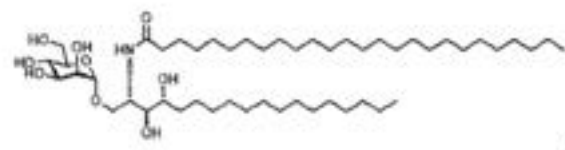
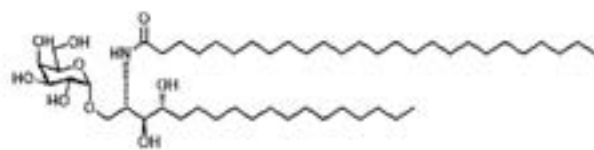
[0055] In some embodiments, X_4 is $-R_3-O-R_4$, wherein R_3 and R_4 are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C_{1-6} alkyl, halogen, C_{1-6} alkyl halogen, and C_{1-6} alkoxy.

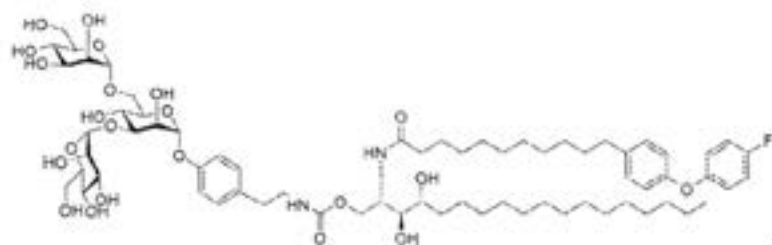
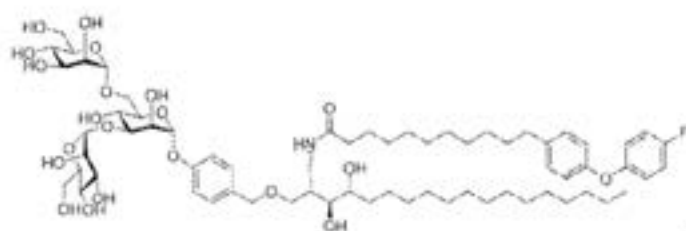
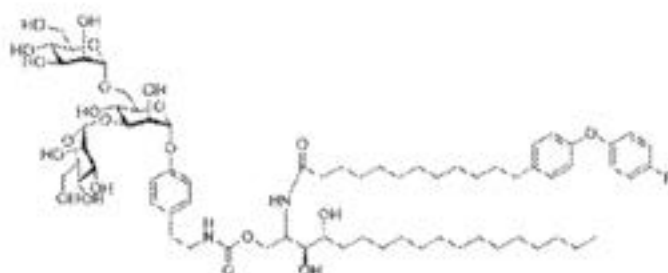
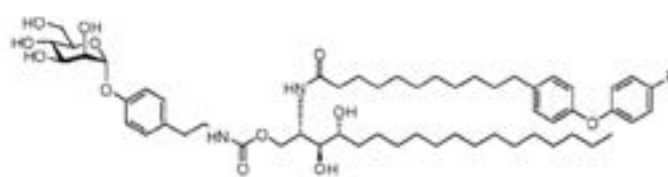
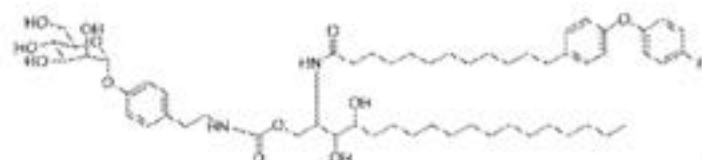
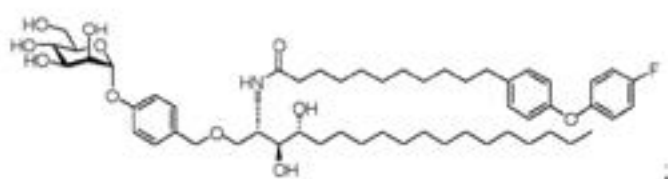
[0056] In certain embodiments, X_4 is selected from the group consisting of:

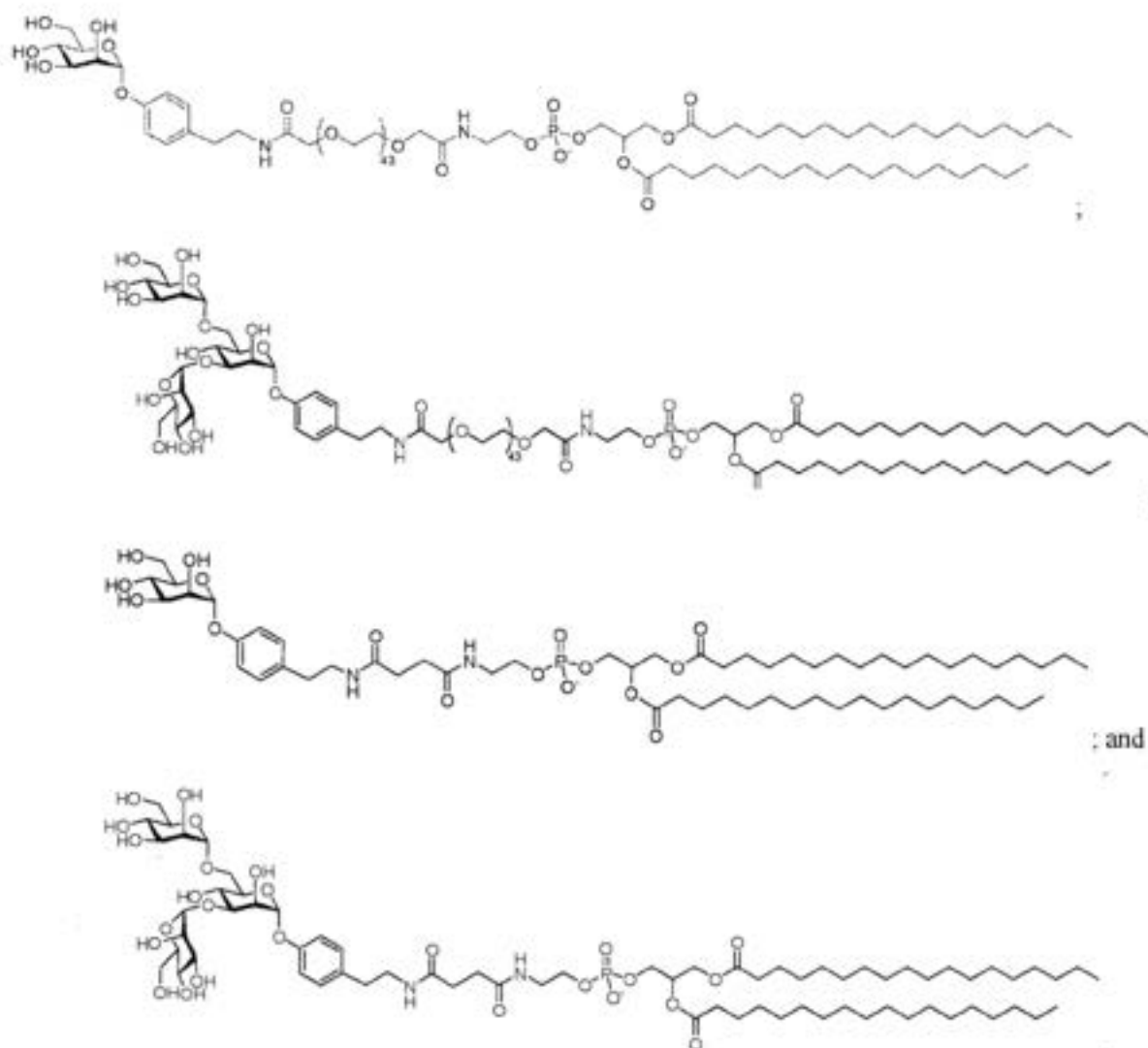


[0057] **Exemplary Compound of the Present Disclosure**

[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).







[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.

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[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-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-

hydroxybutyl)azanediy)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, 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 helper 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, (3 β ,5 β)-3-hydroxycholelan-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 R₁ 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
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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'-O 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*

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[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

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desired specificity or affinity. For example, the R₁ 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

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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 $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ 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, C₁₋₆ 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, C₂₋₆ 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, C₂₋₆ 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, bicyclic, 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.

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[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 $\text{—S(O)}_2\text{—}$. 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 $\text{—S(O)}_2\text{—}$. 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, benzothieryl, 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. ^1H and ^{13}C NMR spectra were recorded on Bruker 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 $\text{DMSO}-d_6$ at δ 2.5 and 39.51 for ^1H 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. IMPACTTM 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 chromelcon software. Transmission electron microscopy (TEM) images were obtained by a FEI Tecnai G2 F20 S-Twin.

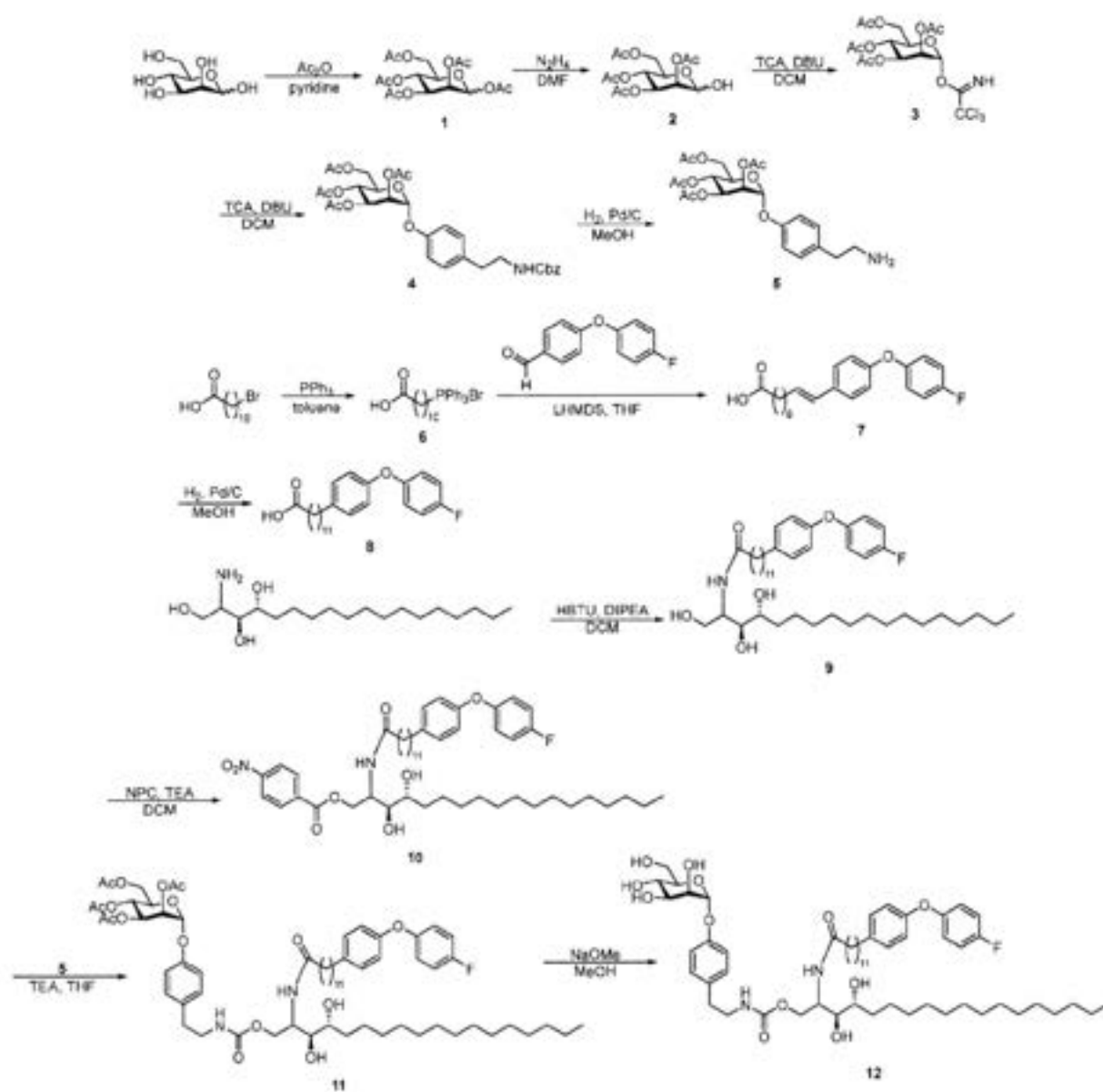
[0160] The chemical materials and methods described herein apply to all examples described in the present disclosure.

[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.

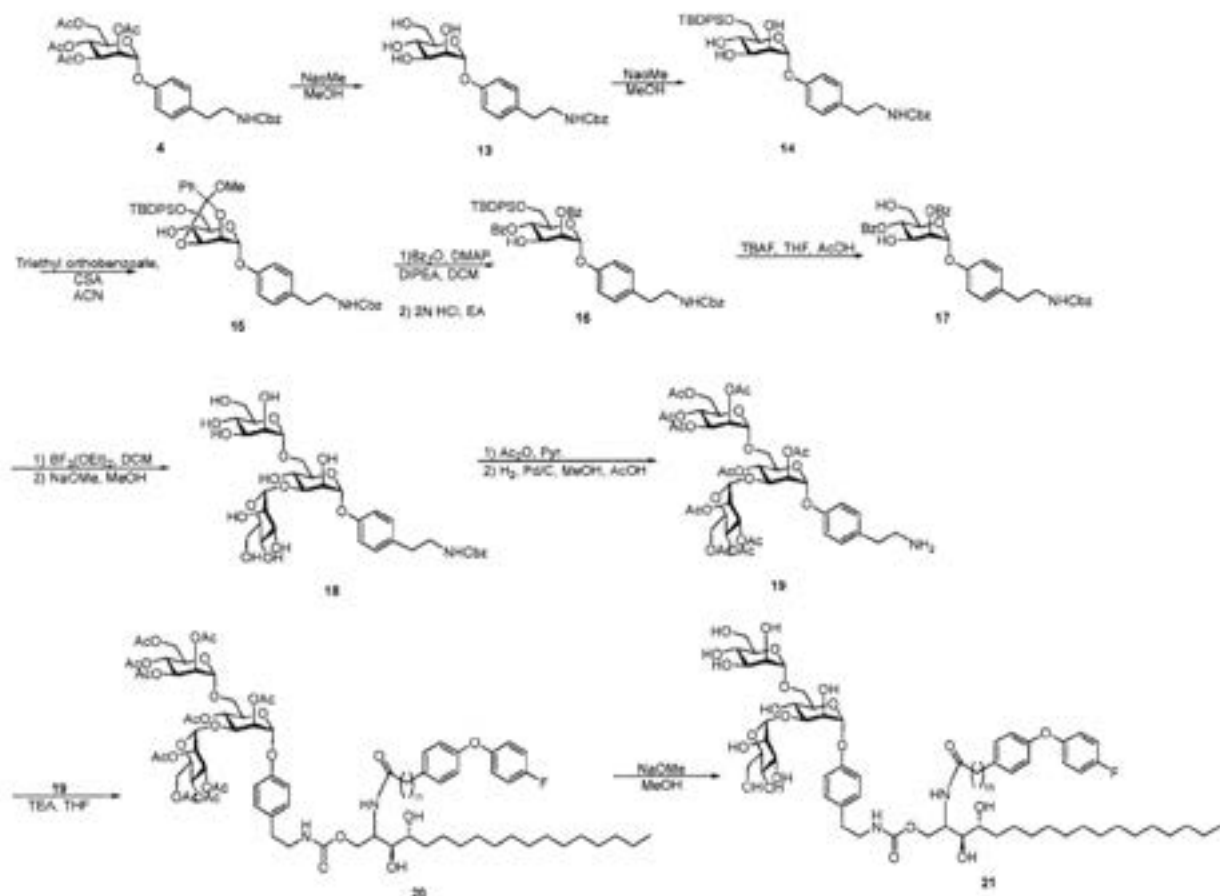
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[0163] Scheme 1



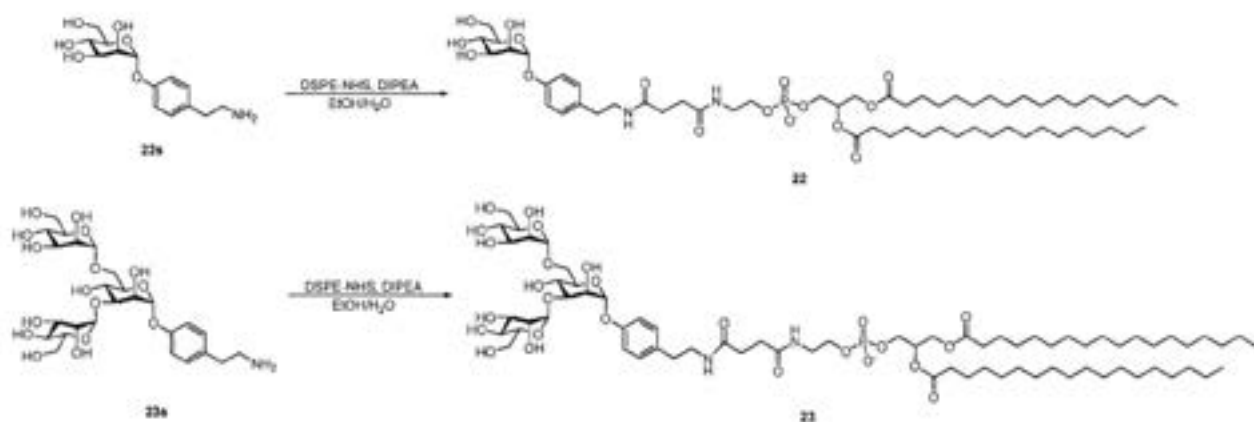
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[0164] Scheme 2



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691

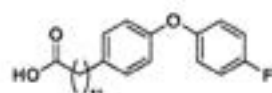
[0165] Scheme 3



[0166] Compounds 1 to 5

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

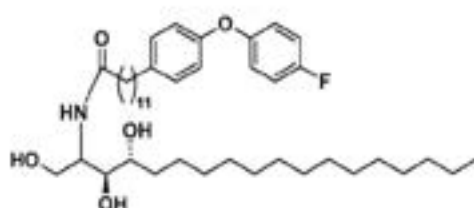
[0168] Compound 8



[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 MgSO₄. 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 H₂ 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%).

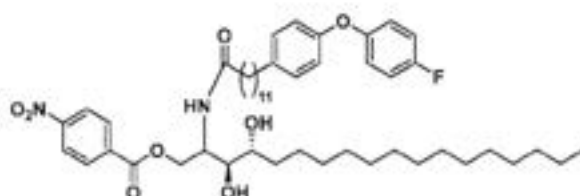
[0170] Compound 9

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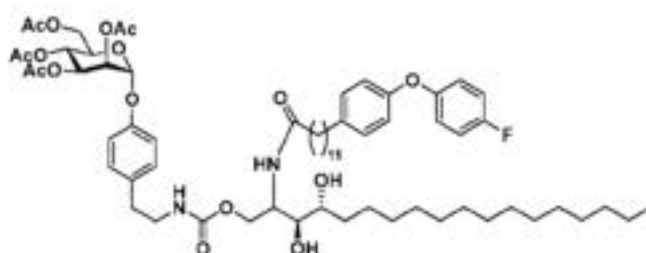
[0171] Compound 9. Compound 8 (1 mmol) in THF (10 mL) was added EDC (1.5 mmol), HOBT (1.5 mmol), DMAP (0.1 mmol), trimethylamine (2 mmol), and phytosphingosine (1.2 mmol), and the resulting solution was stirred under nitrogen at rt for 12 h. The solvent was then removed by evaporation, followed by extraction with EA/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. 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/H₂O. The collected organic layer was washed with

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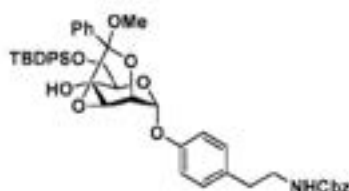
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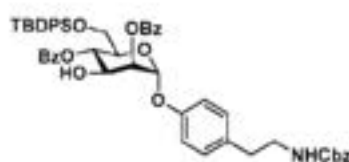
MeOH. After stirring at room temperature for 10 min, the solvent was removed under reduced pressure to give a dry residue that was purified by column chromatography with MeOH/DCM (1/10) to give compound **14** (82%).

[0182] **Compound 15**



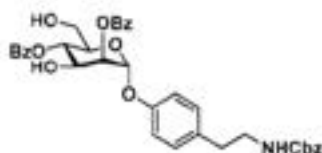
[0183] To a solution of compound **14** (1 mmol) and a catalytic amount of CSA (0.1 mmol) in CH₃CN (20 mL) was added trimethyl orthobenzoate (3 mmol) at room temperature under atmospheric pressure of nitrogen. After stirring for 30 min, Et₃N was added to quench the reaction, and the resulting mixture 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/H₂O. The collected organic layer was washed with ice-cold saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound **16** (71%).

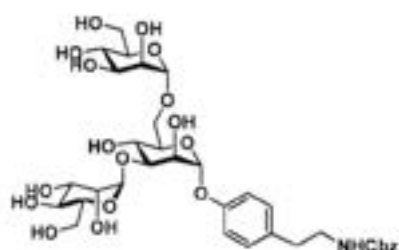
[0186] **Compound 17**



[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 NaHCO₃(aq), water, and brine, dried with anhydrous MgSO₄, and concentrated under reduced pressure. The dry residue was purified by column chromatography with EA/Hex (1/2) to give compound 47 (88%).

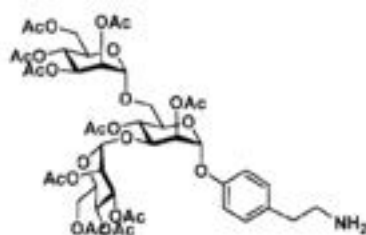
[0188]

[0189] **Compound 18**



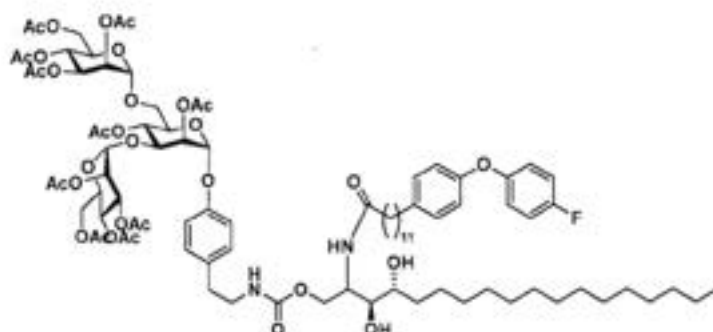
[0190] To a stirred solution of 17 (1 mmol) and 4 Å molecular sieve (0.1 g) in anhydrous DCM (10 mL) was cooled to -40°C and then BF₃(OEt)₂ (0.1 mmol) was added dropwise to the solution. A solution of 3 in anhydrous DCM was added dropwise to the above mixture and stirred for 1 h at -40°C. After that, 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 NaHCO₃ aq. and extracted with DCM. The organic layer was dried with MgSO₄ 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 H₂O as eluent to obtain a pure trisaccharide. The compound was lyophilized to dryness to give compound 18 (39%).

[0191] **Compound 19**



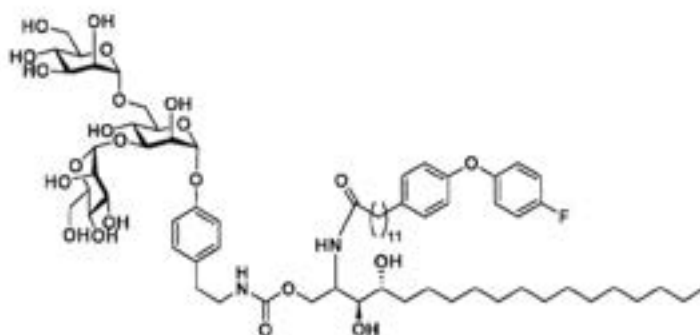
[0192] Compound **13** (1 mmol) in MeOH was added NaOMe (0.2 eq), and the resulting solution was stirred under nitrogen at rt for 2 h. The mixture was neutralized by IR-120 and then filtered and concentrated to dryness in vacuo.

[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/H₂O. The collected organic layer was washed with saturated NaHCO₃(aq), water and brine, and dried over MgSO₄. The crude product was purified by column chromatography on silica gel (EA/Hex 1:1+10% MeOH) to yield **20**.

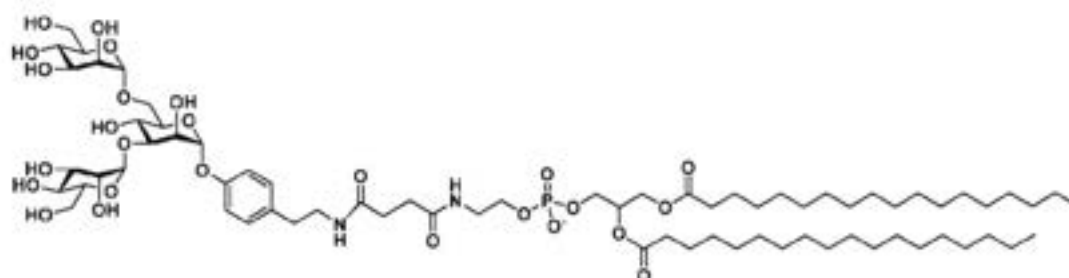
[0195] **Compound 21**



[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).

CCCCCCCCCCCCCCCC(=O)OCCOP(=O)(=O)OCCNC(=O)CCCNC(=O)Cc1ccc(OCC2C(C(C(C(C2O)O)O)O)O)cc1

[0199] Compound 23



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

[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 (%)
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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 μ M 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 μ M 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 °C 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 (BioLegend) 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-BMDCs, 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*

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[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, 1×10^6 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°C 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 CaCl₂, 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 tetramethylbenzidine (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 K_d 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 CO₂ 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

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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 AlamarBlue 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 titrating 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 H₂SO₄. 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.

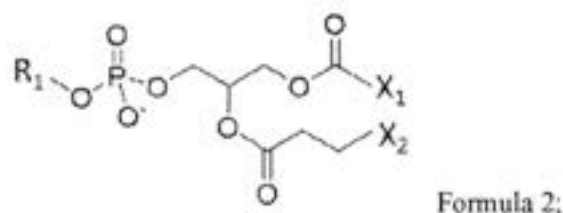
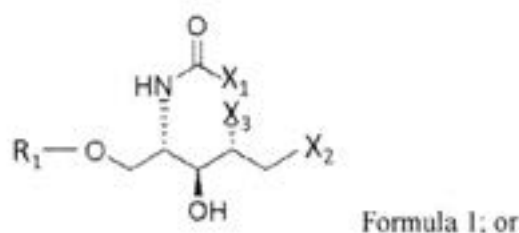
[0237] Furthermore, it was observed that LNP carrying wild-type SARS-CoV-2 spike protein-encoding mRNA ("WT LNP") and LNP carrying mRNA encoding a low-sugar modified spike protein ("low-sugar LNP") induced comparable IgG titers against wide-type viruses, the WT LNP had lower IgG

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:



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.

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[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.

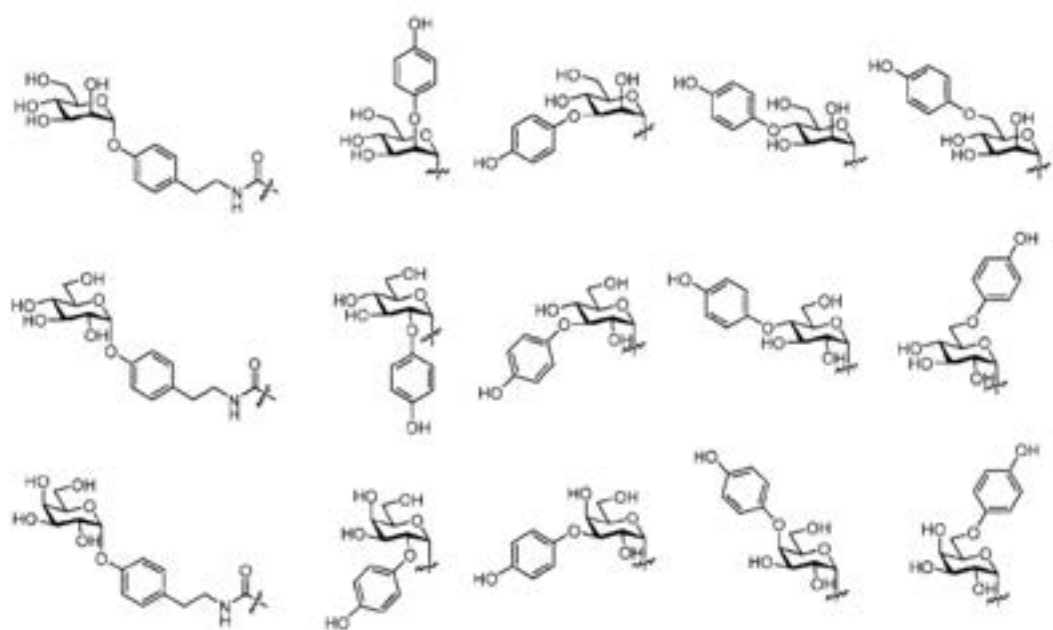
[0251] Embodiment 12. The compound of embodiment 11, wherein the glycosyl group comprises 1 to 6 substituents, wherein the substituent is C_{1-6} alkyl, C_{1-6} alkenyl, halogen, C_{1-6} alkyl halide, C_{1-6} alkoxy, amine, nitro, C_{1-6} alkyl amine, amide, azido, aryl, cycloalkyl, heterocycloalkyl, 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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ alkyl amine, amide, azido, carboxyl, hydroxyl, aryl, cycloalkyl, heterocycloalkyl, or a substituted version thereof, or a combination thereof.

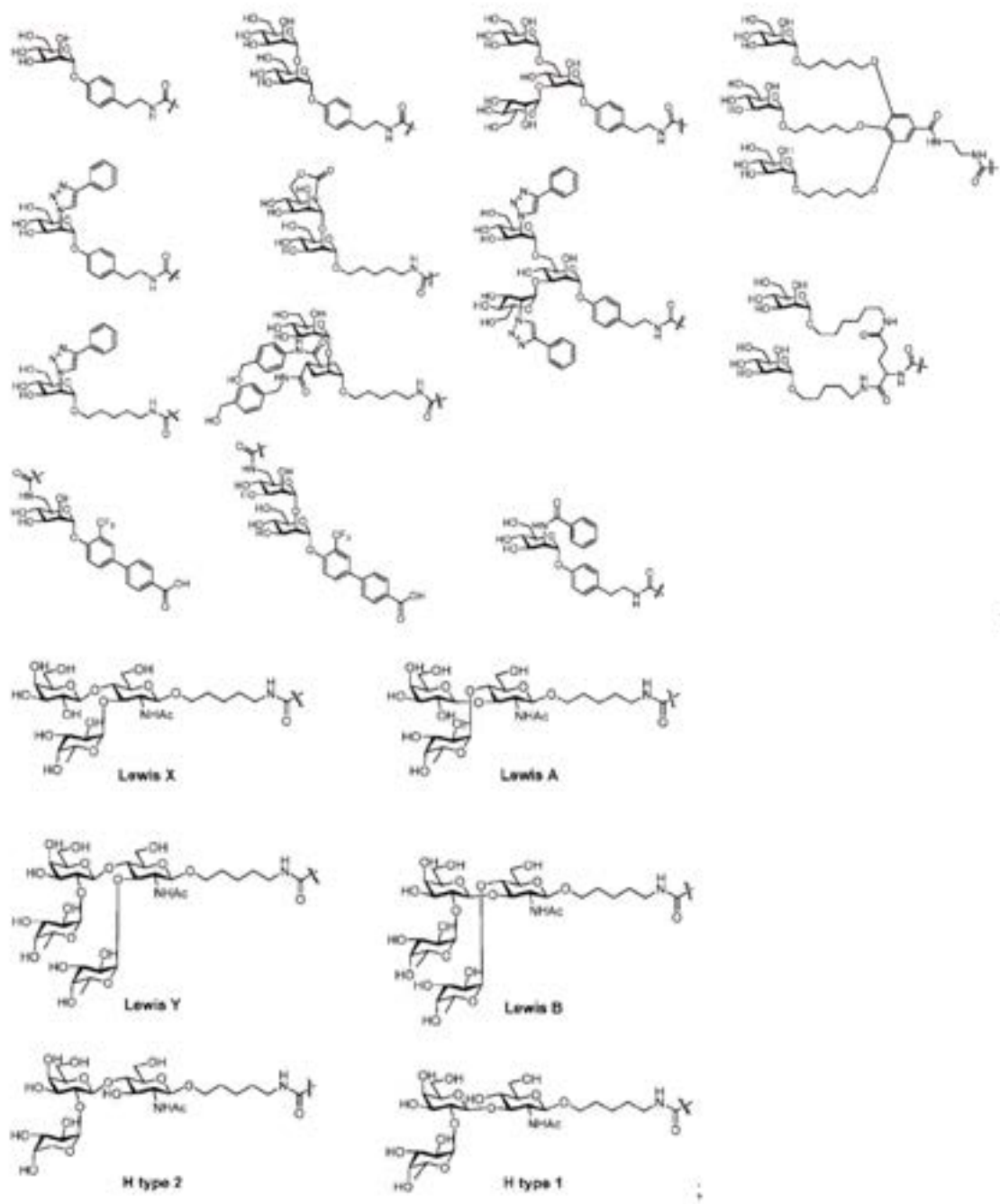
[0253] Embodiment 14. The compound of embodiment 12 or embodiment 13, wherein the substituent of the glycosyl group is a substituted or non-substituted aryl, optionally the substituent of the glycosyl group is a phenyl substituted with OH, CH₃, NH₂, CF₃, OCH₃, F, Br, Cl, NO₂, N₃, or a combination thereof.

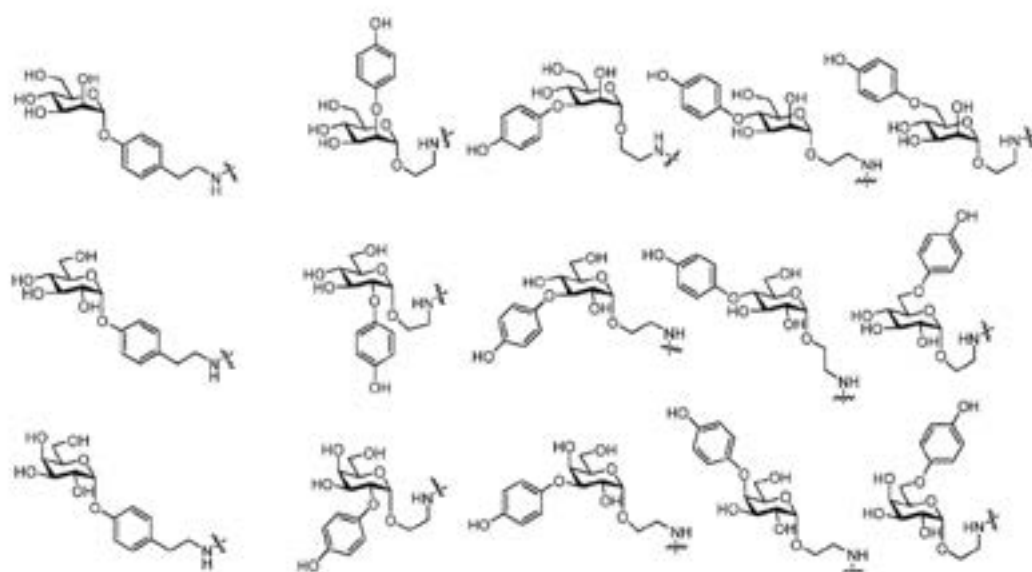
[0254] Embodiment 15. The compound of embodiment 12 or embodiment 13, wherein the heterocycloalkyl comprises 1 to 3 heteroatoms, selected from the group consisting of O, S, and N.

[0255] Embodiment 16. The compound of any one of embodiments 1 to 15, wherein R₁ is selected from the group consisting of:



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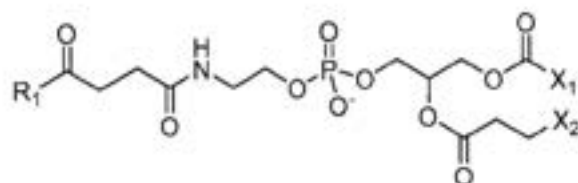




[0256] Embodiment 17. The compound of any one of embodiments 1 to 16, wherein the compound is of Formula 1.

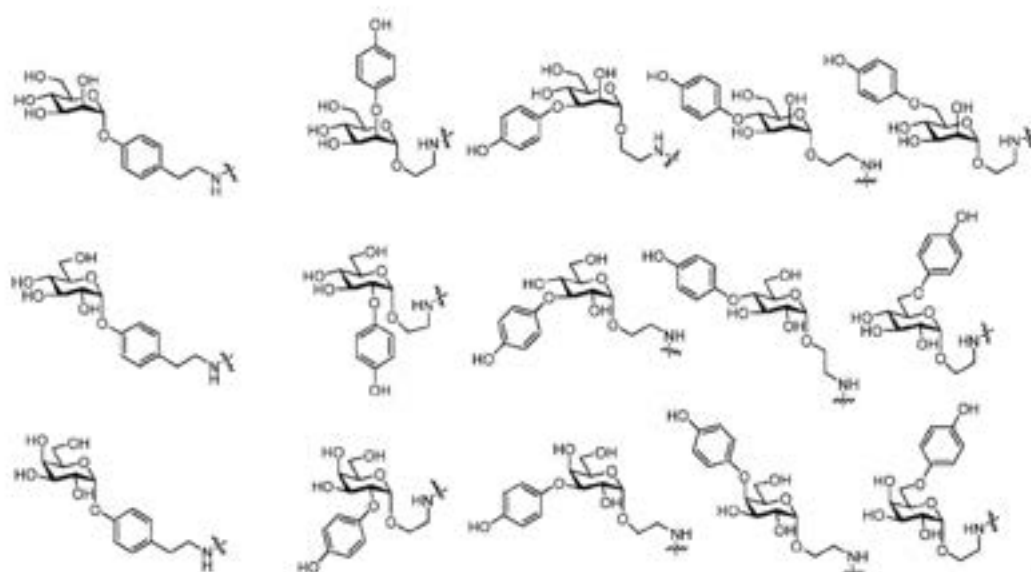
[0257] Embodiment 18. The compound of any one of embodiments 1 to 16, wherein the compound is of Formula 2.

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



Formula 3; and

wherein R₁ 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_{4-30} 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.

[0261] Embodiment 22. The compound of embodiment 21, wherein 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.

[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 C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halogen, and C₁₋₆ alkoxy.

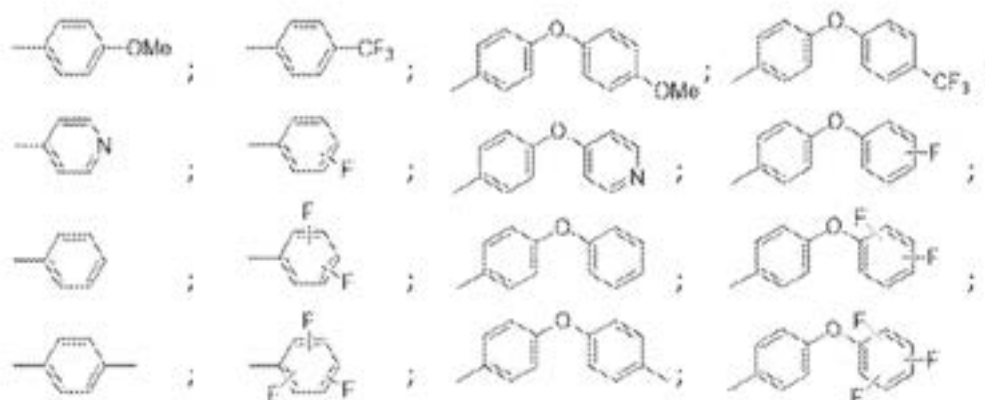
[0264] Embodiment 25. The compound of embodiment 24, wherein the substituent is CH₃, CF₃, F, or OCH₃.

[0265] Embodiment 26. The compound of embodiment 24 or embodiment 25, wherein X₄ comprises 1 to 3 substituents.

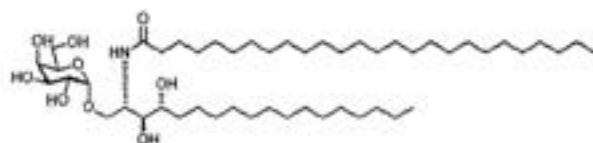
[0266] Embodiment 27. The compound of any one of embodiments 24 to 26, wherein X₄ is -R₃-O-R₄, wherein R₃ and R₄ are each independently aryl, heterocyclic group, cycloalkyl, heterocycloalkyl, each comprising 0 to 6 substituents selected from the group consisting of C₁₋₆ alkyl, halogen, C₁₋₆ alkyl halogen, and C₁₋₆ alkoxy.

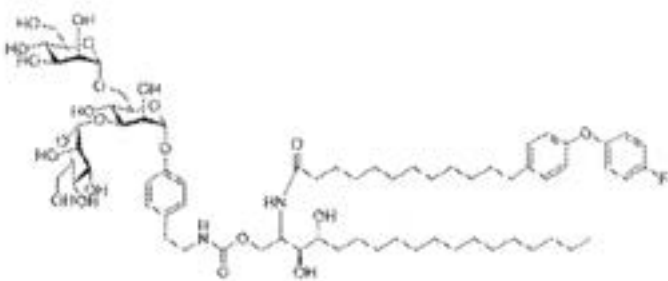
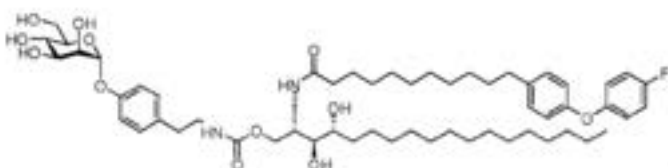
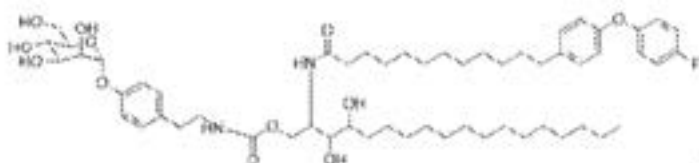
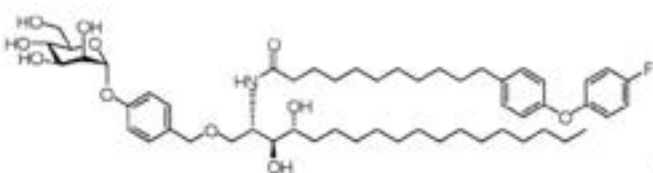
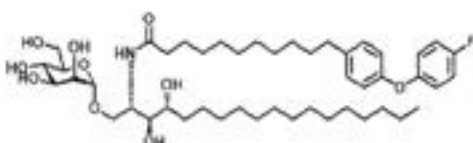
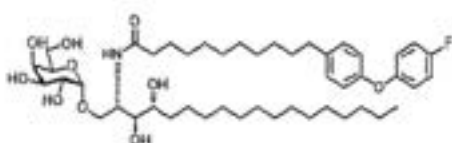
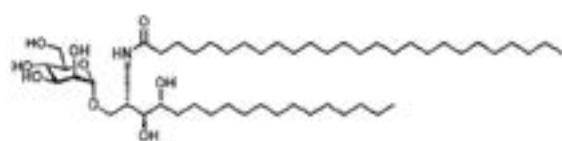
[0267] Embodiment 28. The compound of any one of embodiments 1 to 27, wherein one of X₁ and X₂ is C₁₅₋₃₀ alkyl, and the other one is -(CH₂)_nX₄.

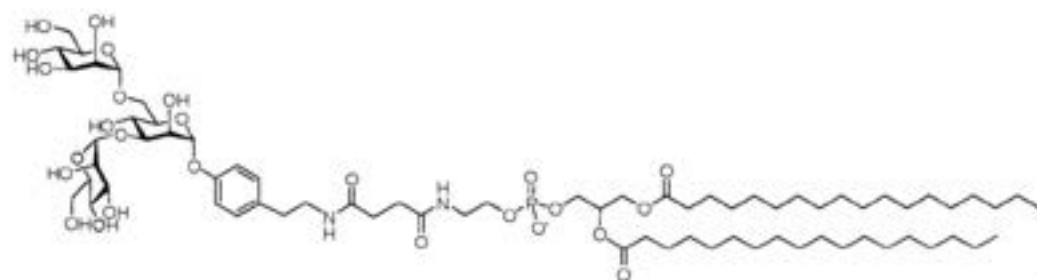
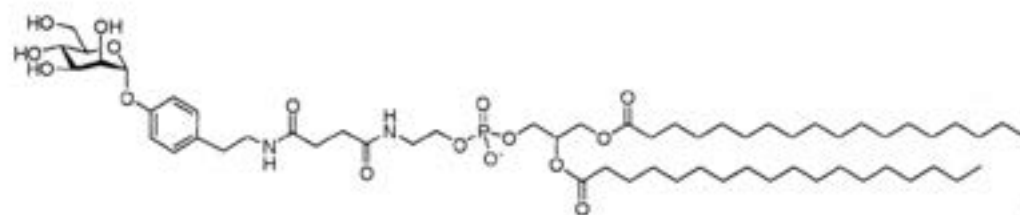
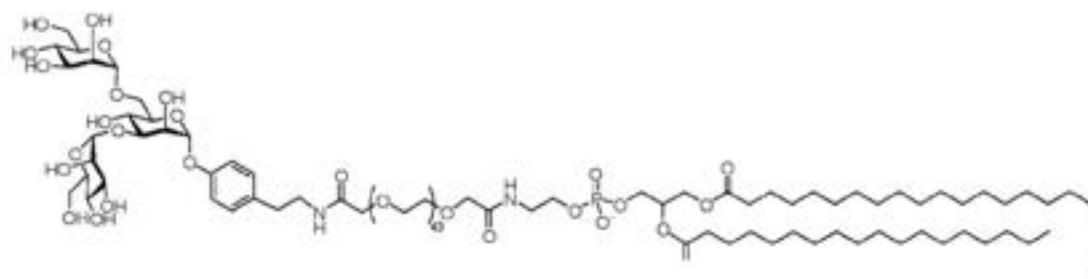
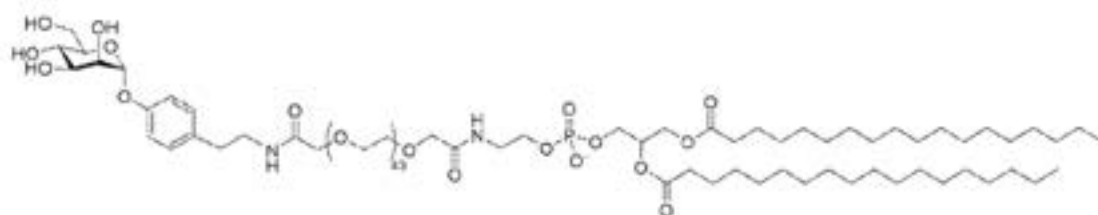
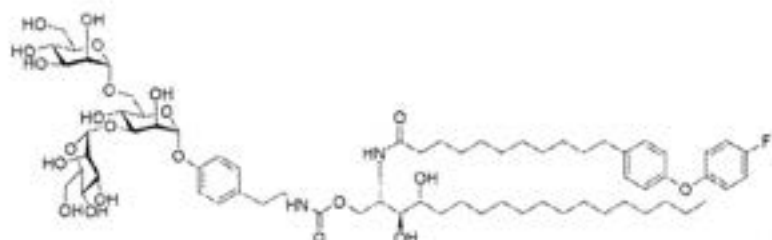
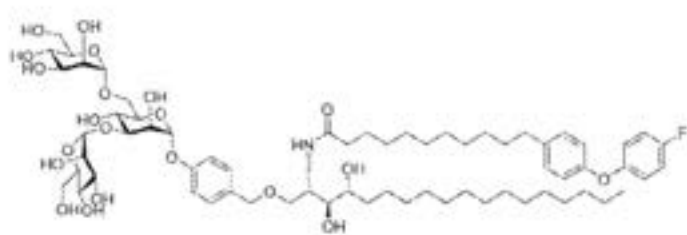
[0268] Embodiment 29. The compound of any one of embodiments 1 to 28, wherein X₄ is selected from the group consisting of:



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







[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-undecoxylhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, 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, (3 β ,5 β)-3-hydroxycholesterol-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-

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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.

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[0289] Embodiment 50. The lipid nanoparticle of embodiment 49, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, 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, (3 β ,5 β)-3-hydroxycholesterol-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.

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[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.

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[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-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, 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.

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[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, (3 β ,5 β)-3-hydroxycholelan-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 μ g to 1000 μ g.

[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.

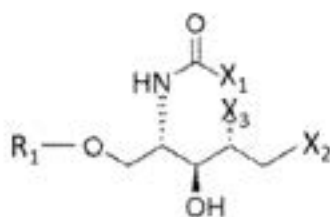
[0354] Embodiment 115. The method of any one of embodiments 107 to 114, wherein the lipid nanoparticle is administered in an initial dose and followed by one, two, three, four, five, or more booster doses.

[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.

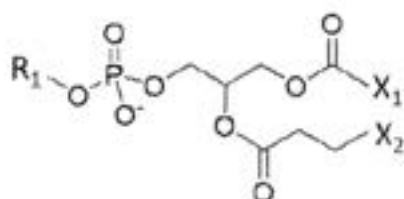
[0356] Embodiment 117. The method of any one of embodiments 107 to 116, wherein the effective amount ranges from about 5 μ g to 1000 μ g.

What is claimed is:

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

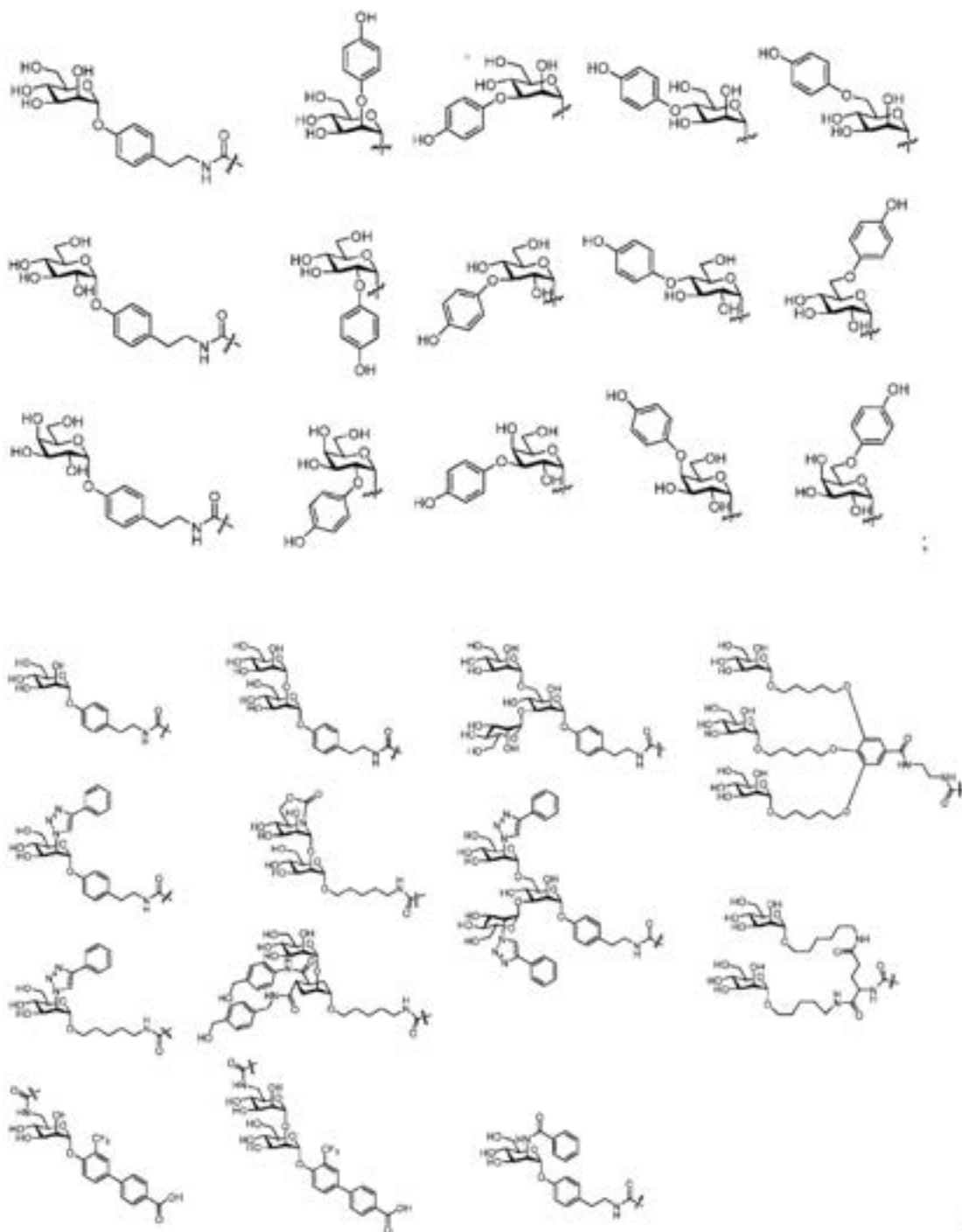
wherein X_3 is hydrogen, C_{1-6} alkyl, or hydroxyl.

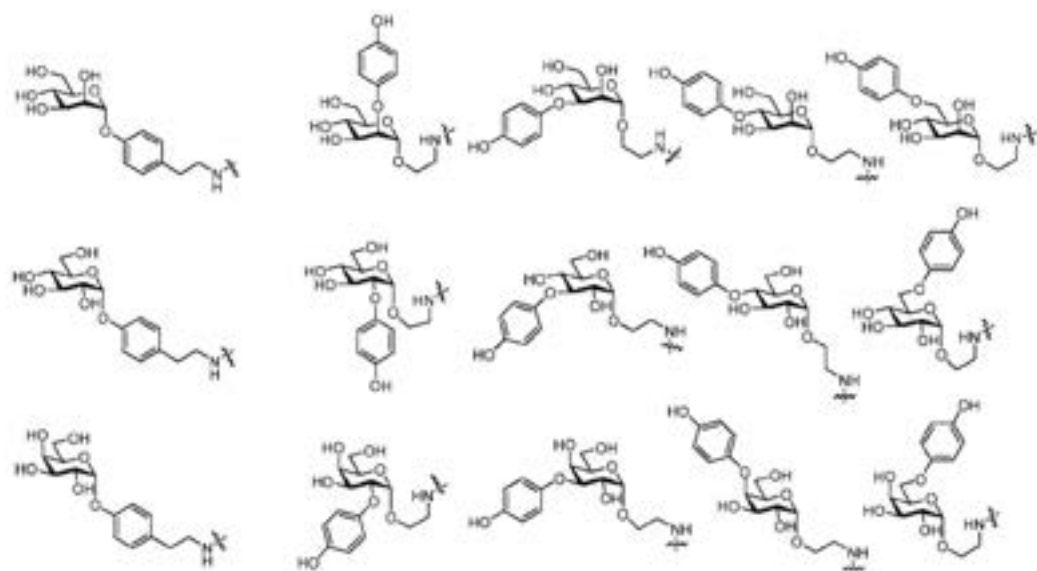
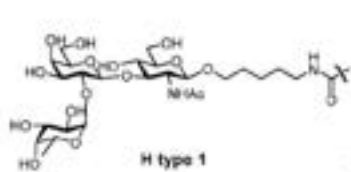
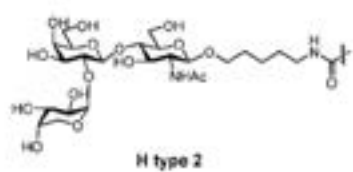
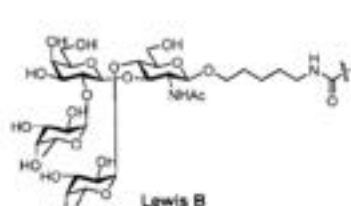
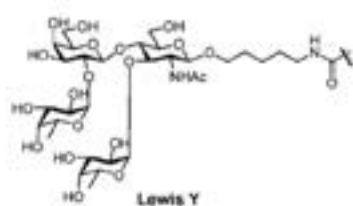
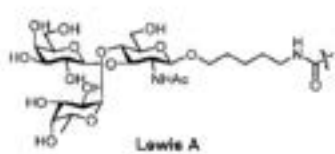
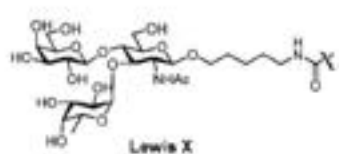
2. The compound of claim 1, wherein R_1 comprises a formula of R_2-R_A , wherein R_2 is the substituted or non-substituted glycosyl group, and R_A is an attachment group, and wherein the attachment group comprises an aryl, an alkyl, an amide, an alkylamide, a substituted version thereof, a combination thereof, or a covalent bond.
3. The compound of claim 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 claim 3, wherein R_A further comprises a polyethylene glycol (PEG) moiety having 2 to 72 (OCH_2CH_2) subunits.
5. The compound of claim 1, wherein the glycosyl group comprises mannoside, fucoside, or a combination thereof.
6. The compound of claim 1, wherein the glycosyl group comprises a mono-mannoside, a di-mannoside, or a tri-mannoside.
7. The compound of claim 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

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halide, C₁₋₆ alkoxy, amine, nitro, C₁₋₆ alkyl amine, amide, azido, aryl, cycloalkyl, heterocycloalkyl, sulfite, or a substituted version thereof, or a combination thereof.

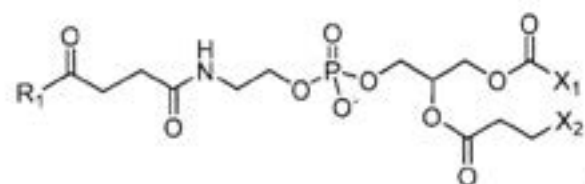
8. The compound of claim 1, wherein R₁ is selected from the group consisting of:





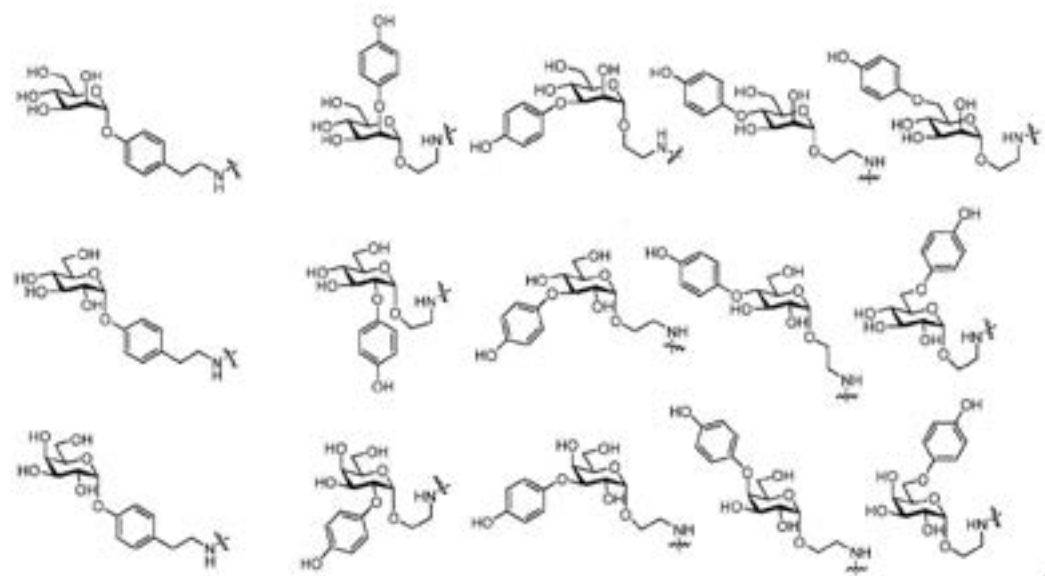
9. The compound of claim 1, wherein the compound is of Formula 1.

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

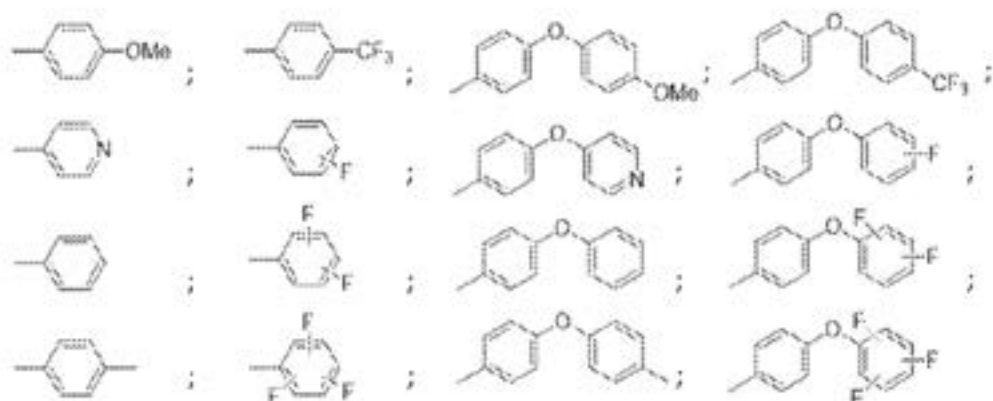


Formula 3; and

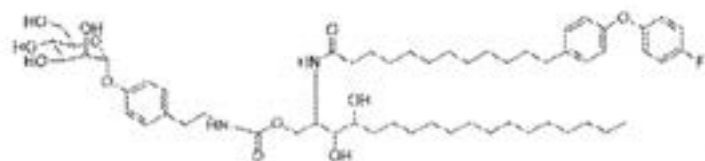
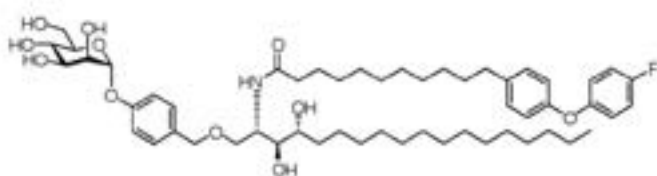
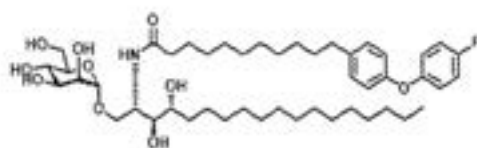
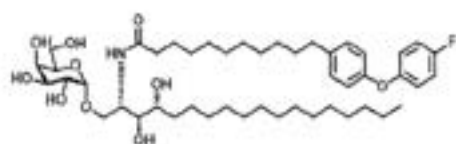
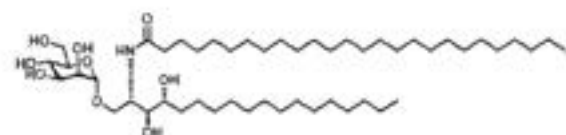
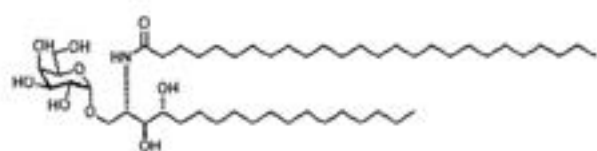
wherein R_1 is selected from the group consisting of:



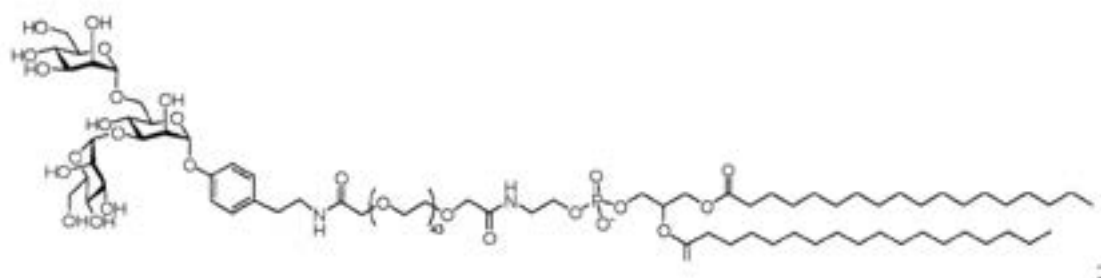
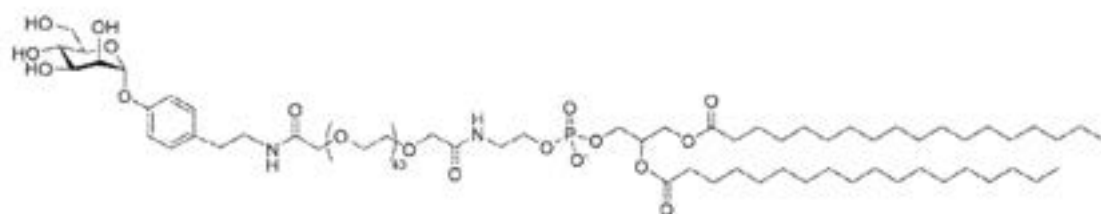
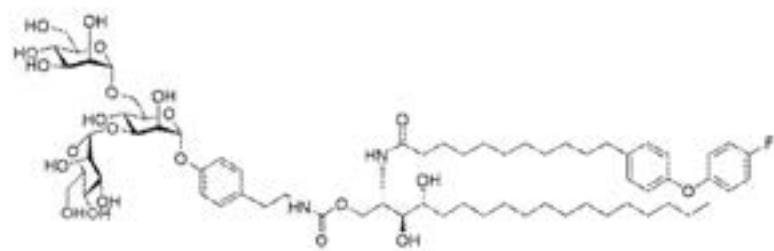
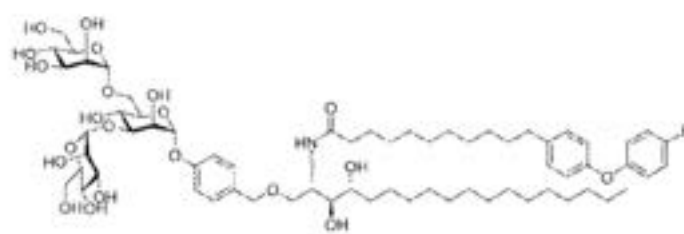
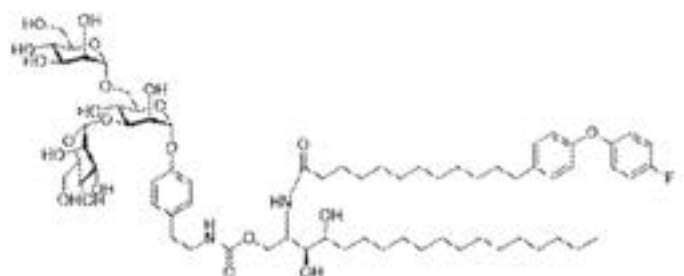
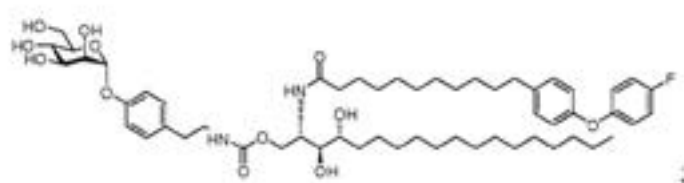
11. The compound of claim 1, wherein at least one of X_1 and X_2 comprises a saturated hydrocarbon chain, comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, or 30 carbons.
12. The compound of claim 1, wherein X_1 and X_2 are each independently hydrogen, 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 claim 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 claim 13, wherein X_4 is $-R_1-O-R_4$, wherein R_1 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 claim 1, wherein X_4 is selected from the group consisting of:

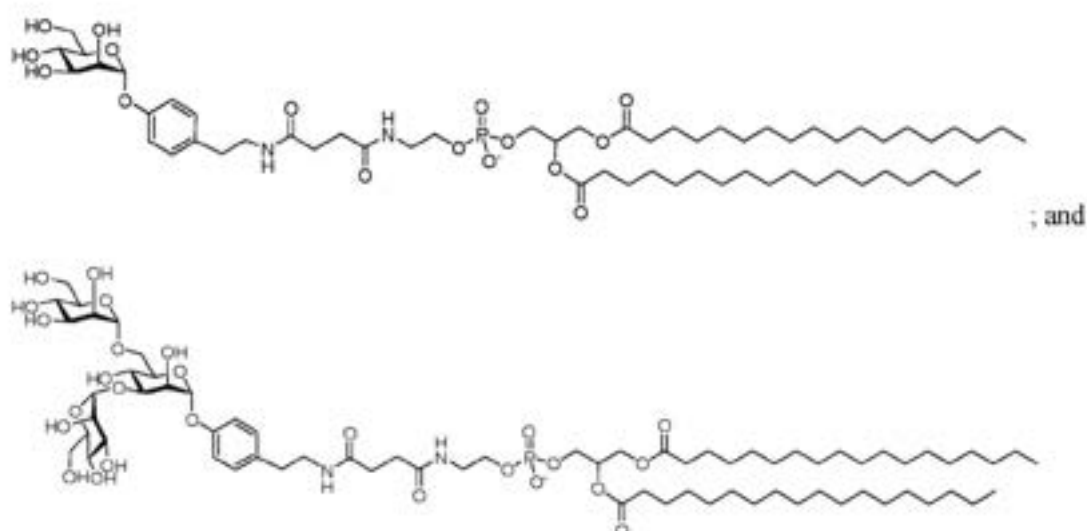


16. The compound of claim 1, selected from the group consisting of:



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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 claims 1 to 16.
18. The lipid nanoparticle of claim 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 claim 17, wherein the membrane is formed via hydrophobic interaction between the plurality of the lipid components.
20. The lipid nanoparticle of claim 19, wherein the ionizable lipid comprises heptadecan-9-yl 8-[2-hydroxyethyl-(6-oxo-6-undecyloxyhexyl)amino]octanoate (SM-102TM), (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315TM, Pfizer), or a combination thereof.
21. The lipid nanoparticle of claim 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 claim 21, wherein the nucleic acid is RNA or DNA encoding a polypeptide.
23. The lipid nanoparticle of claim 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 claim 21, wherein the payload is a first payload, and the membrane further encapsulates a second payload.
25. A formulation, comprising the lipid nanoparticle of claim 17.
26. The formulation of claim 25, comprising 0.01 to 95% (w/w) of the lipid nanoparticle.

27. The formulation of claim 25, wherein the lipid nanoparticle is a first lipid nanoparticle, and the composition further comprises a second lipid nanoparticle.
28. The formulation of claim 25, further comprising an excipient, an adjuvant, or a combination thereof.
29. The formulation of claim 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 claim 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.

ABSTRACT

The present disclosure relates to novel compounds, methods, and cell-targeting formulations, e.g., a lipid nanoparticle (LNP) for targeted delivery to a tissue or a cell type. The compound and formulation provided herein are designed to have a targeting moiety configured to provide selective delivery features for the formulation and a lipid tail for being incorporated into the bilayer membrane of the formed lipid nanoparticle.



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION # PCT/US24/23590	RECEIPT DATE / TIME 04/08/2024 04:49:42 PM Z ET	ATTORNEY DOCKET # A1000-01100PCT-2
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Title of Invention

METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

Application Information

APPLICATION TYPE	International Application (PCT) for filing in the US receiving office	PATENT #	-
CONFIRMATION #	1954	FILED BY	Shannon Warner
PATENT CENTER #	65008649	FILING DATE	-
CUSTOMER #	169053	APPLICANT NAME	ROCK BIOMEDICAL INC.
CORRESPONDENCE ADDRESS	Wade Lin, Prosyla Group PC 530 Technology Dr., Suite 100 Irvine, CA 92618 US	AUTHORIZED BY	Wade Lin

Documents

TOTAL DOCUMENTS: 6

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01100PCT-2_20240405_Request Form_FINAL.pdf	6	-	434 KB
A1000-01100PCT-2_20240405_Request Form_FINAL-P.N.101.CONV.pdf	(1-5) 5	PCT/RO/101 - Request form for new International Application - Conventional	431 KB
A1000-01100PCT-2_20240405_Request Form_FINAL-P.N.101.ANX.pdf	(6-6) 1	PCT/RO/101 Annex (fee calculation sheet)	242 KB

A1000-01100PCT- 2_20240407_Specification_T BF.pdf	87	-		1613 KB
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A1000-01100PCT- 2_20240407_Specification_ TBF-ABST.pdf	(87-87)	1	Abstract	95 KB
● 000-01100PCT- 2_20240407_Drawings_TBF. pdf	10		Drawings-only black and white line drawings	2009 KB

Digest

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If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1610), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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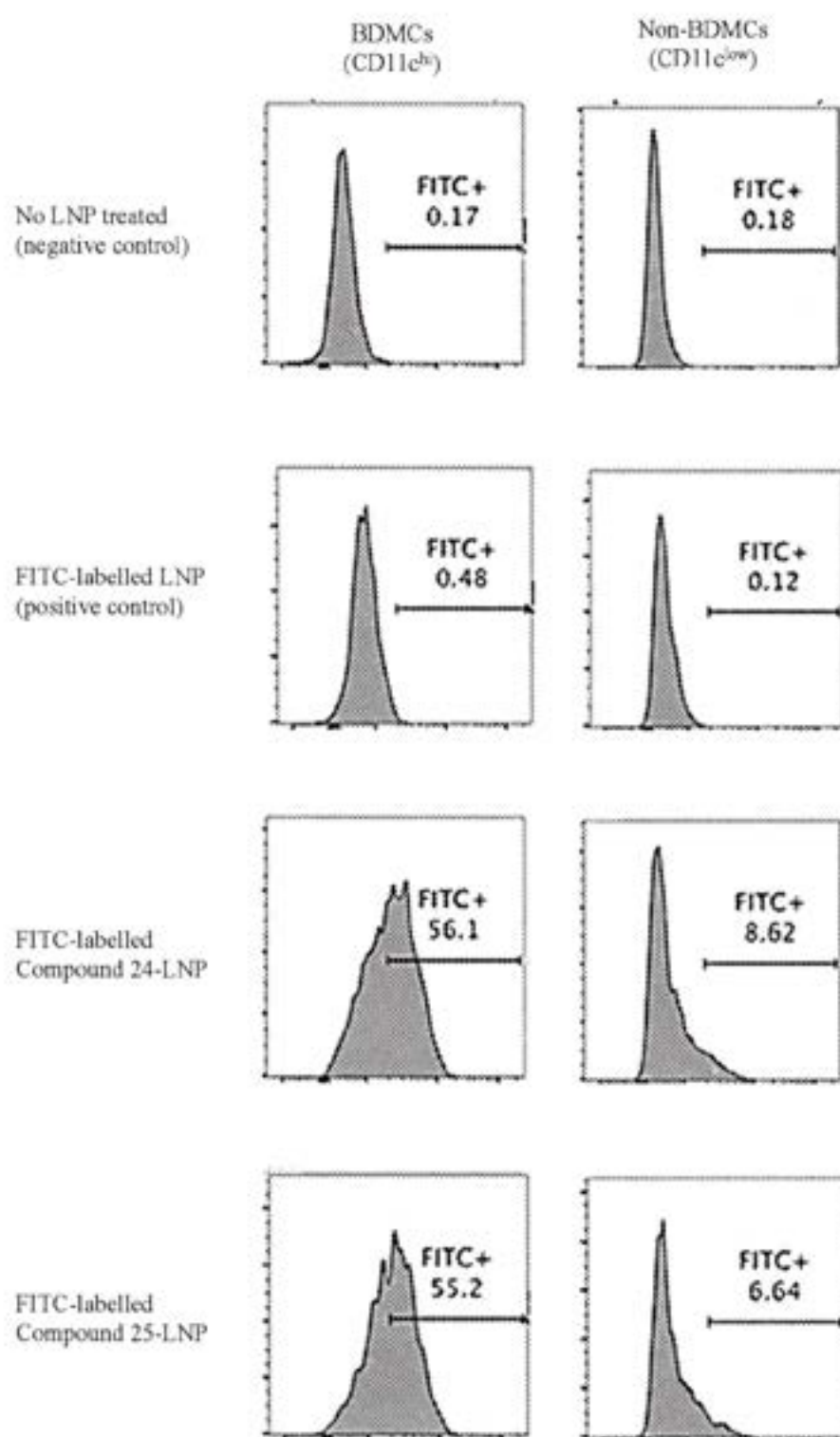


FIG. 1

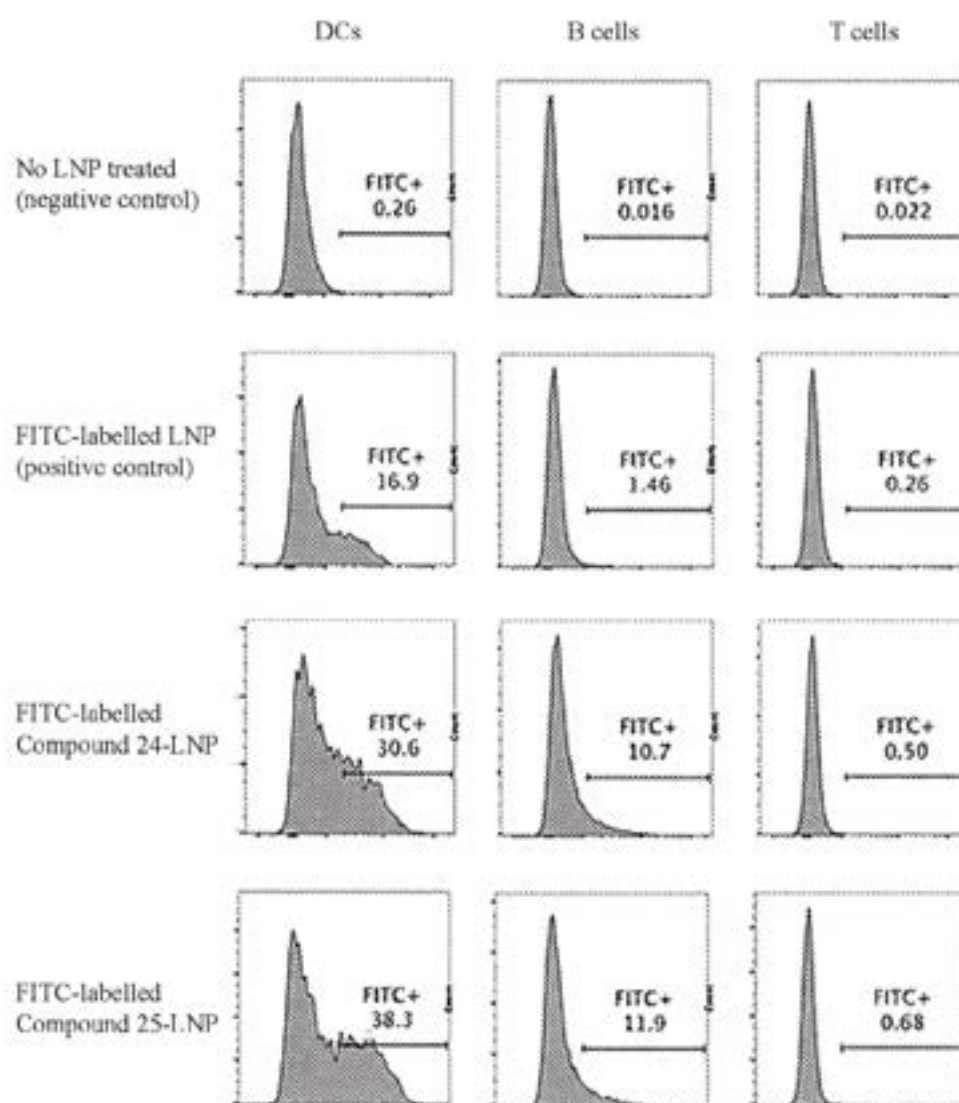


FIG. 2

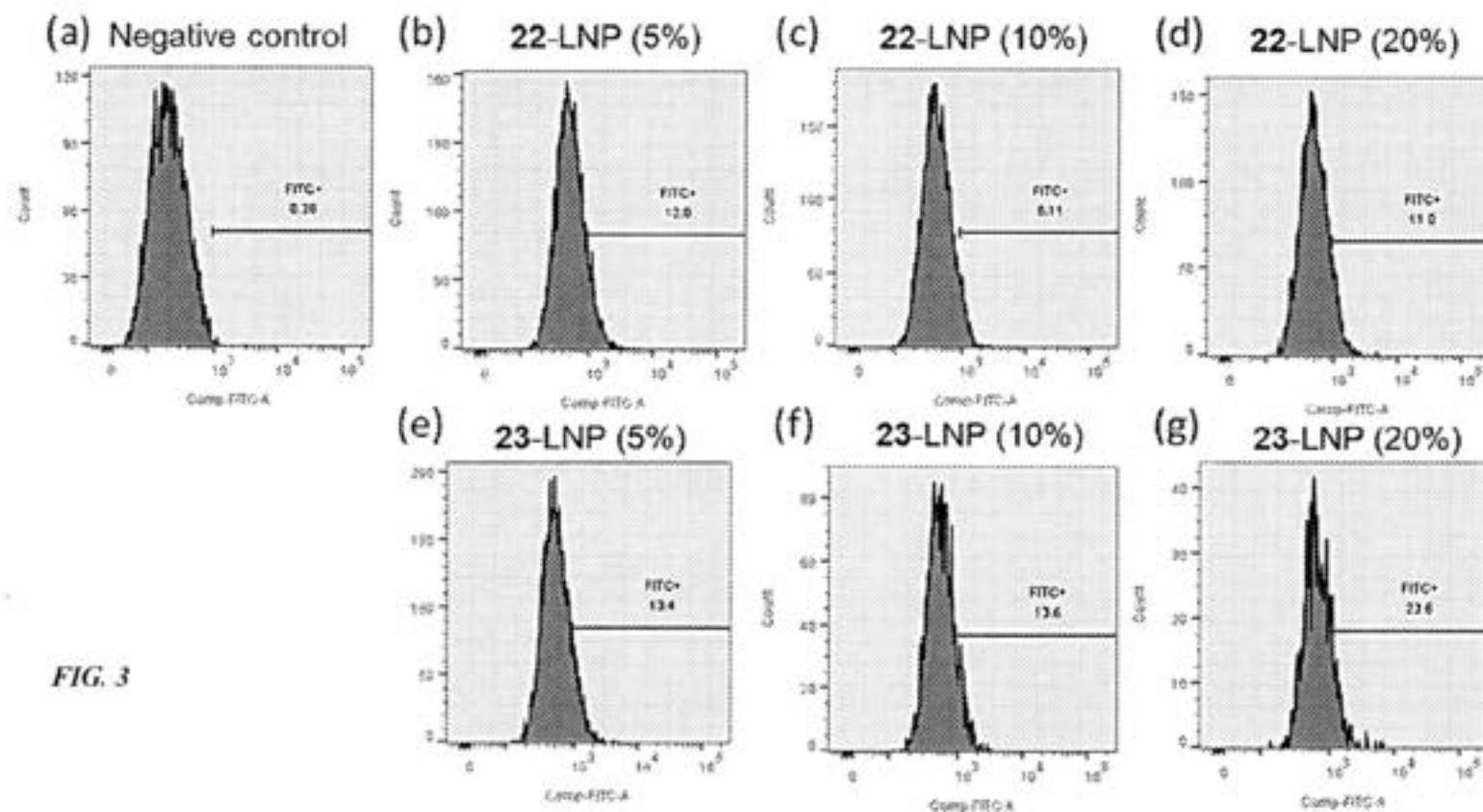


FIG. 3

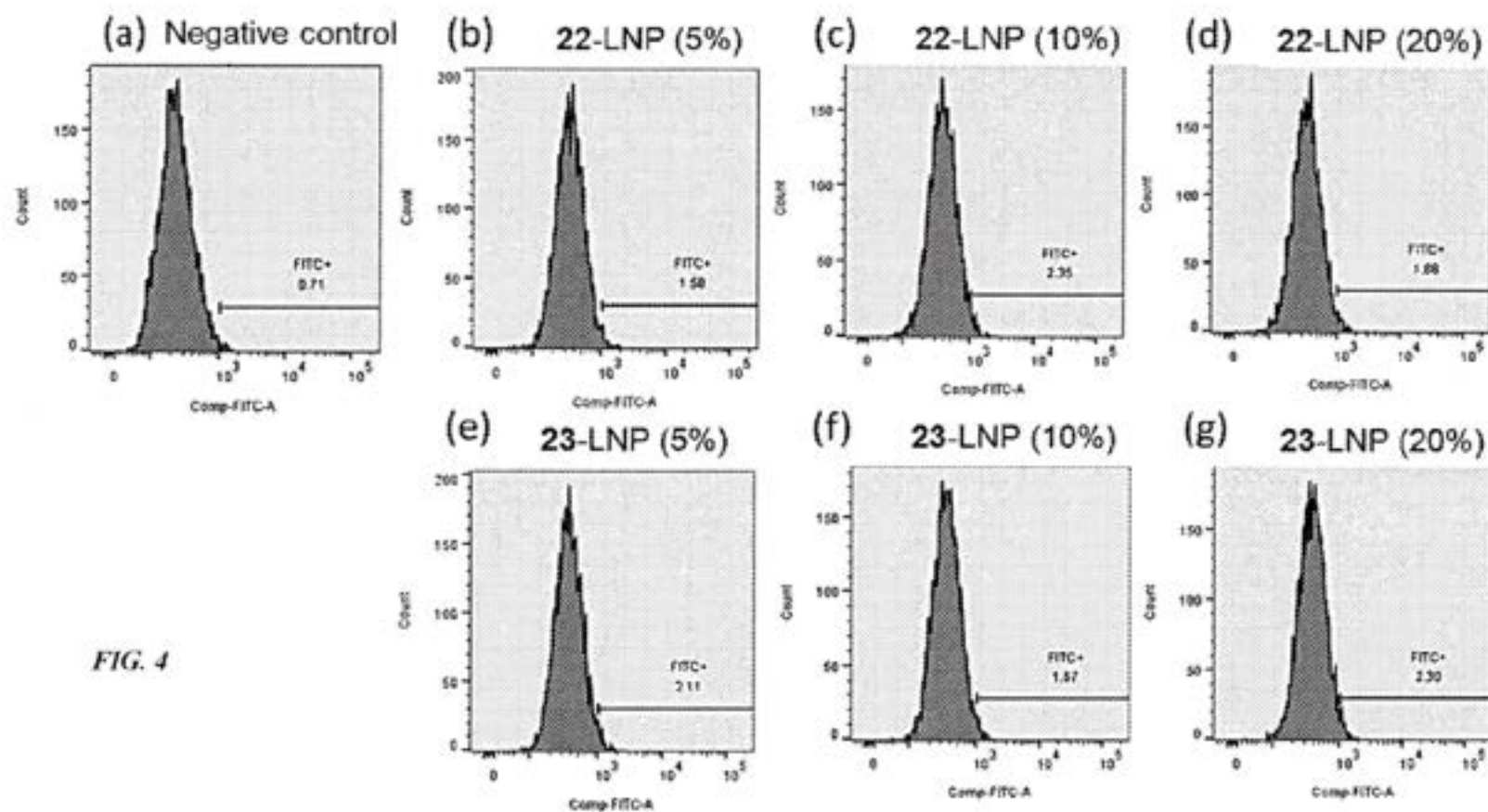


FIG. 4

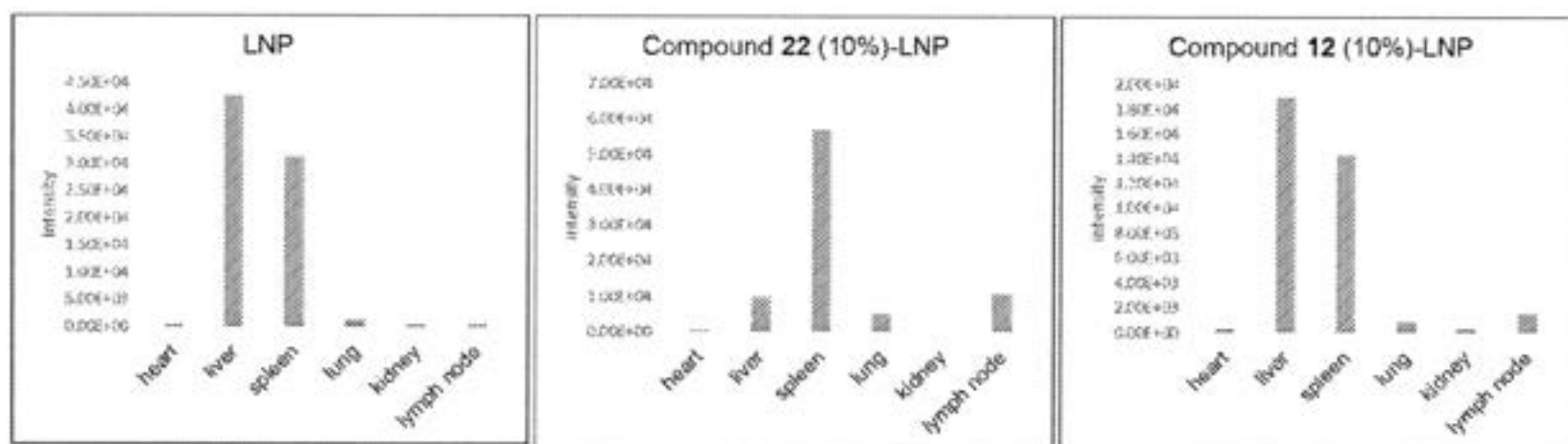


FIG. 5

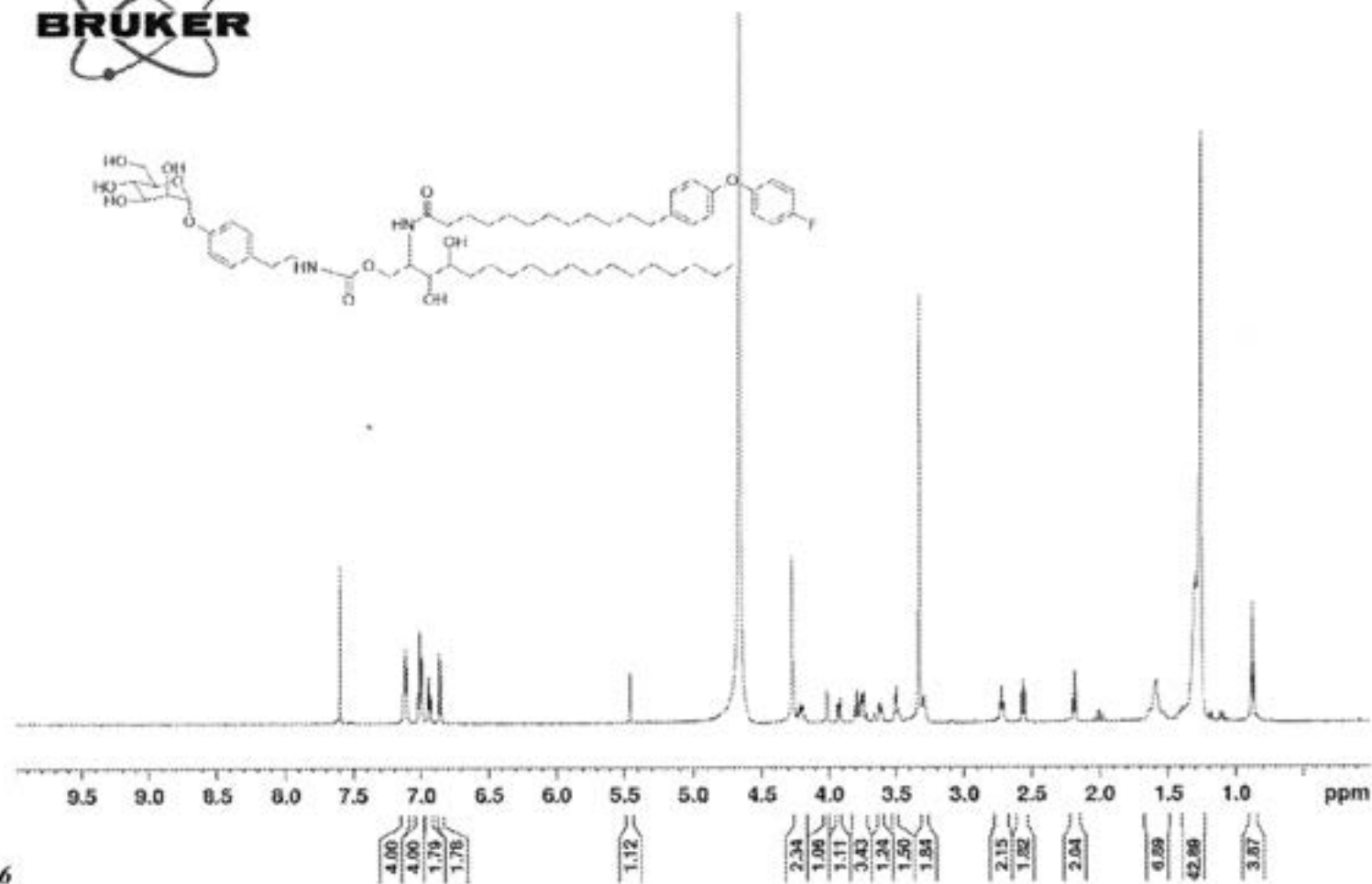
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FIG. 6

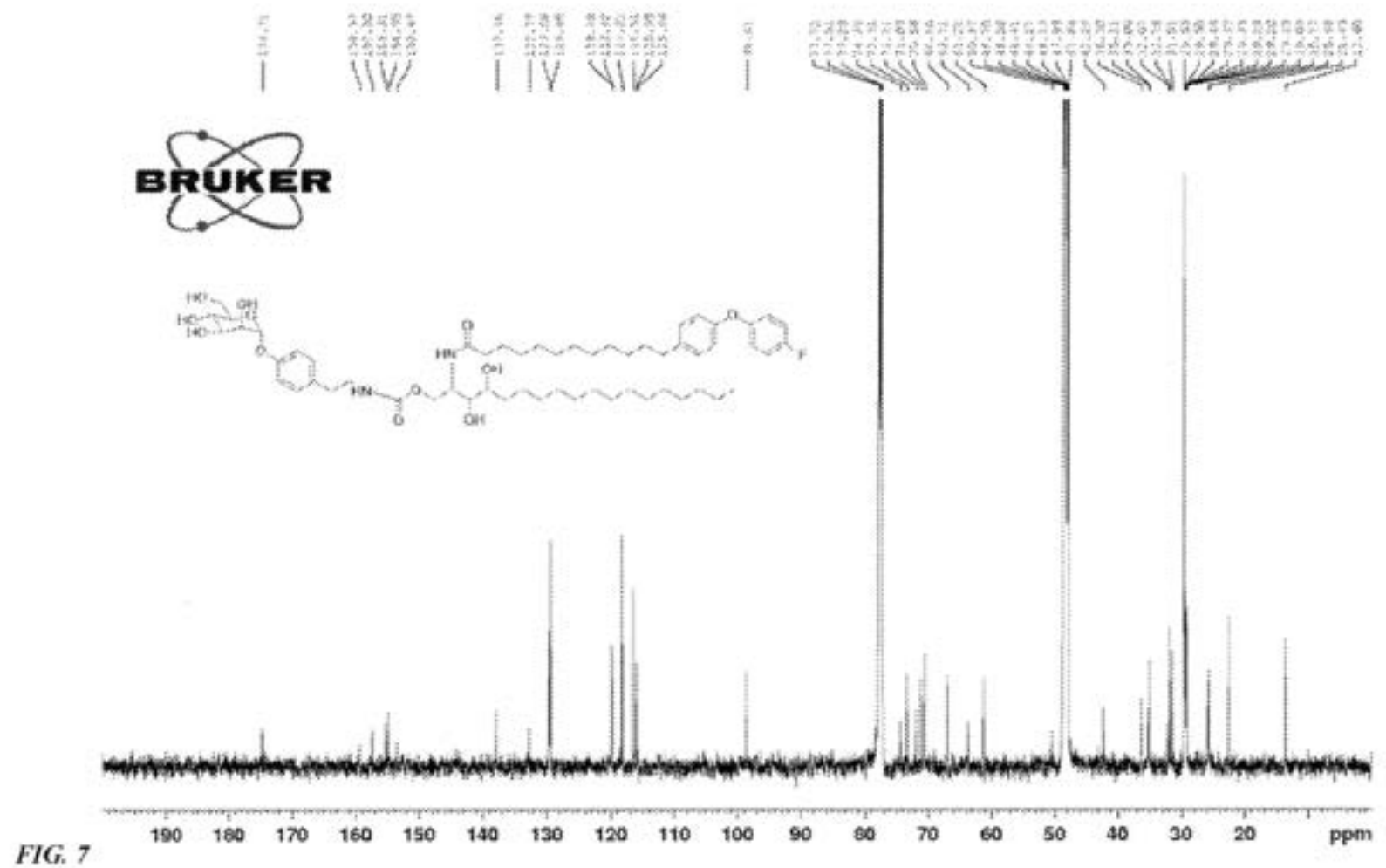


FIG. 7

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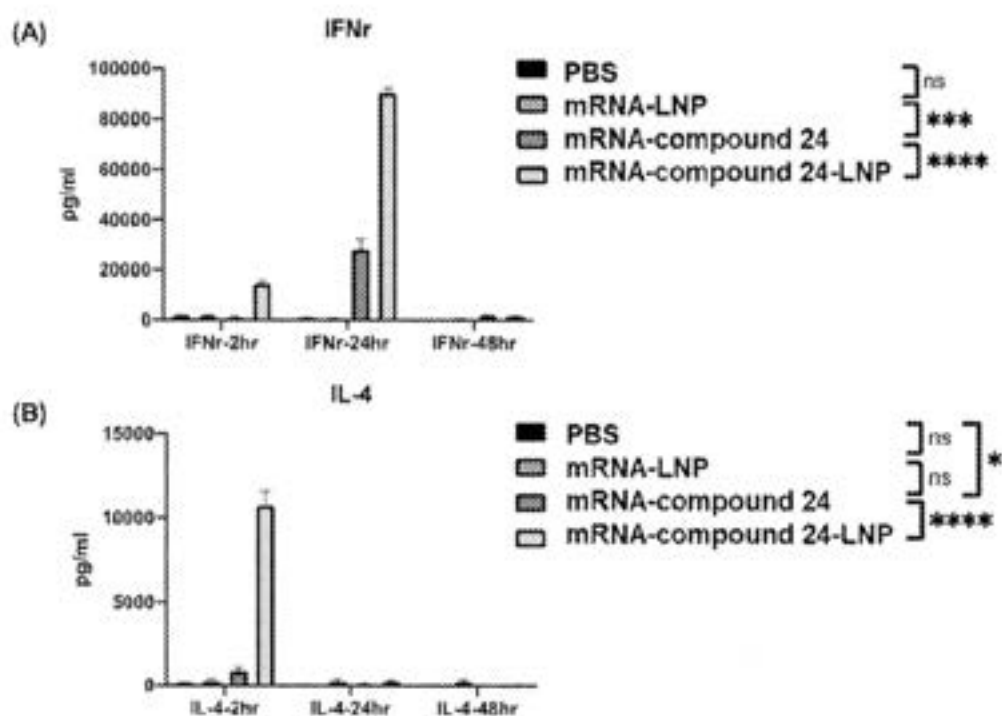


FIG. 8

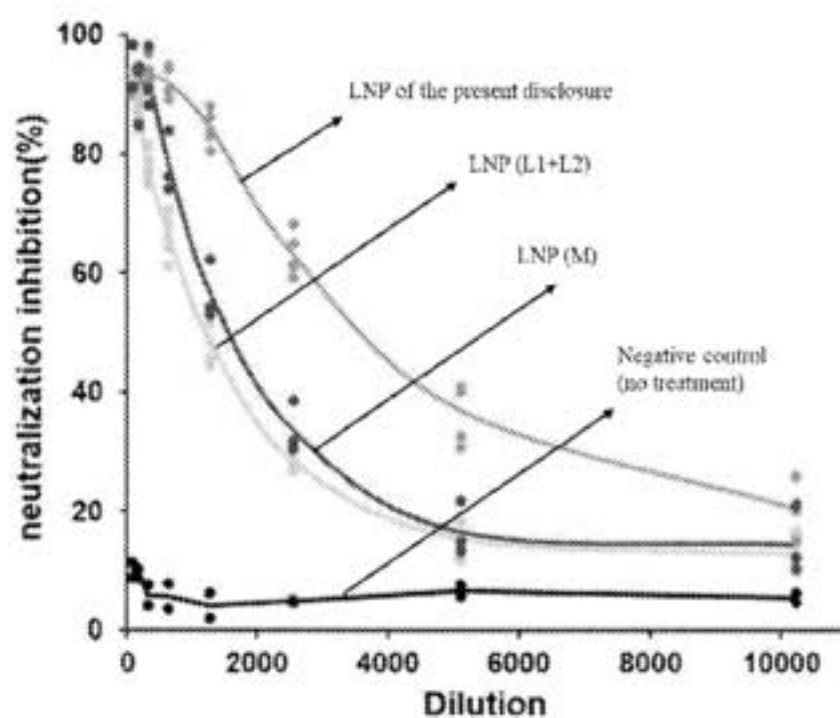


FIG. 9

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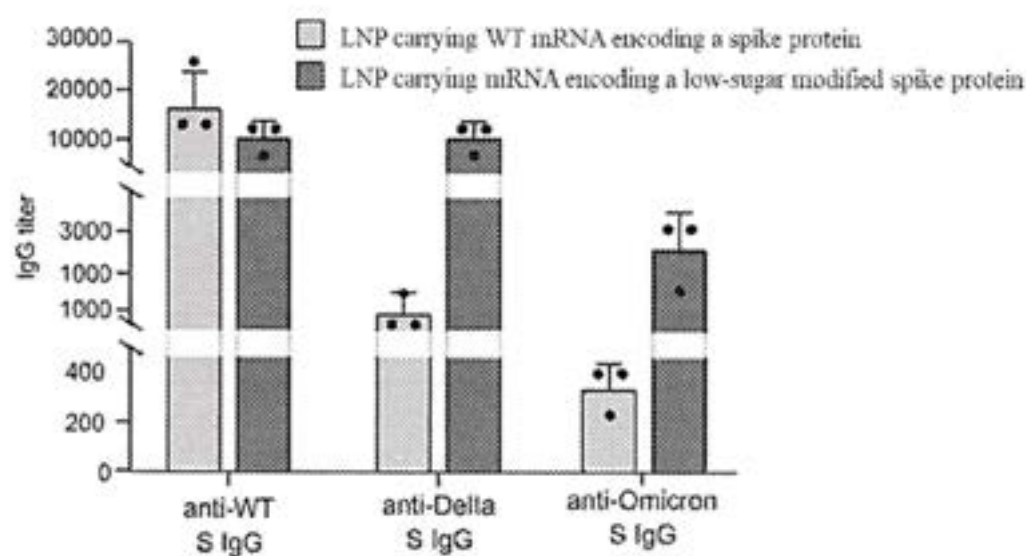


FIG. 10

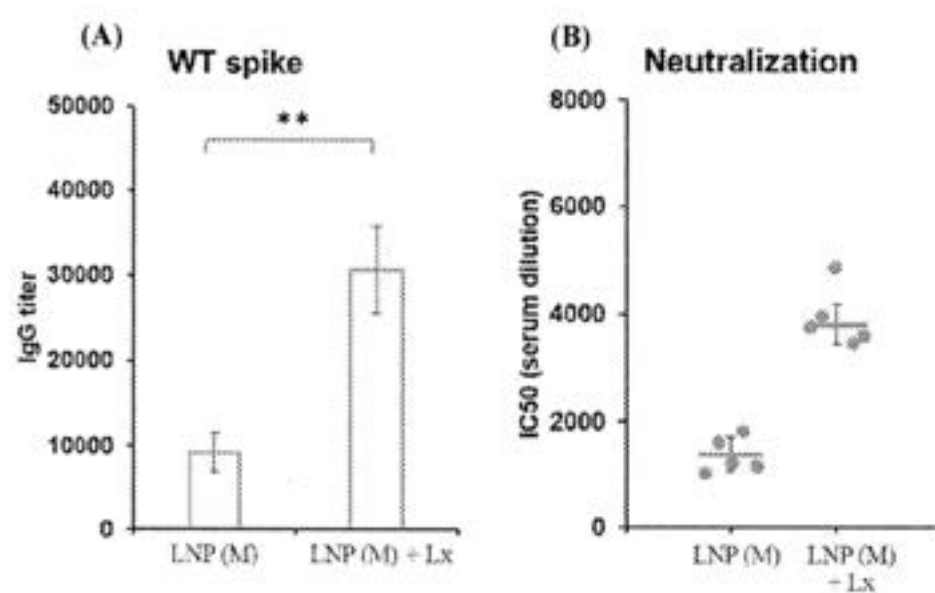


FIG. 11

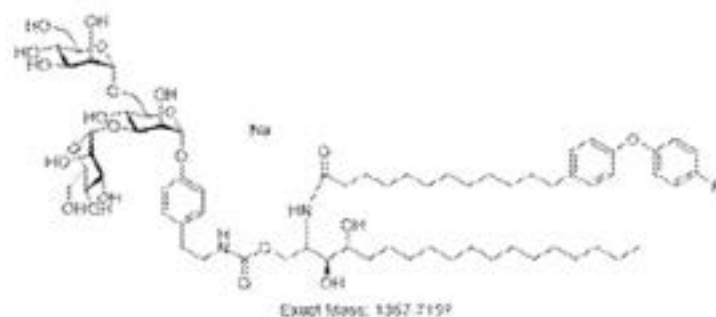
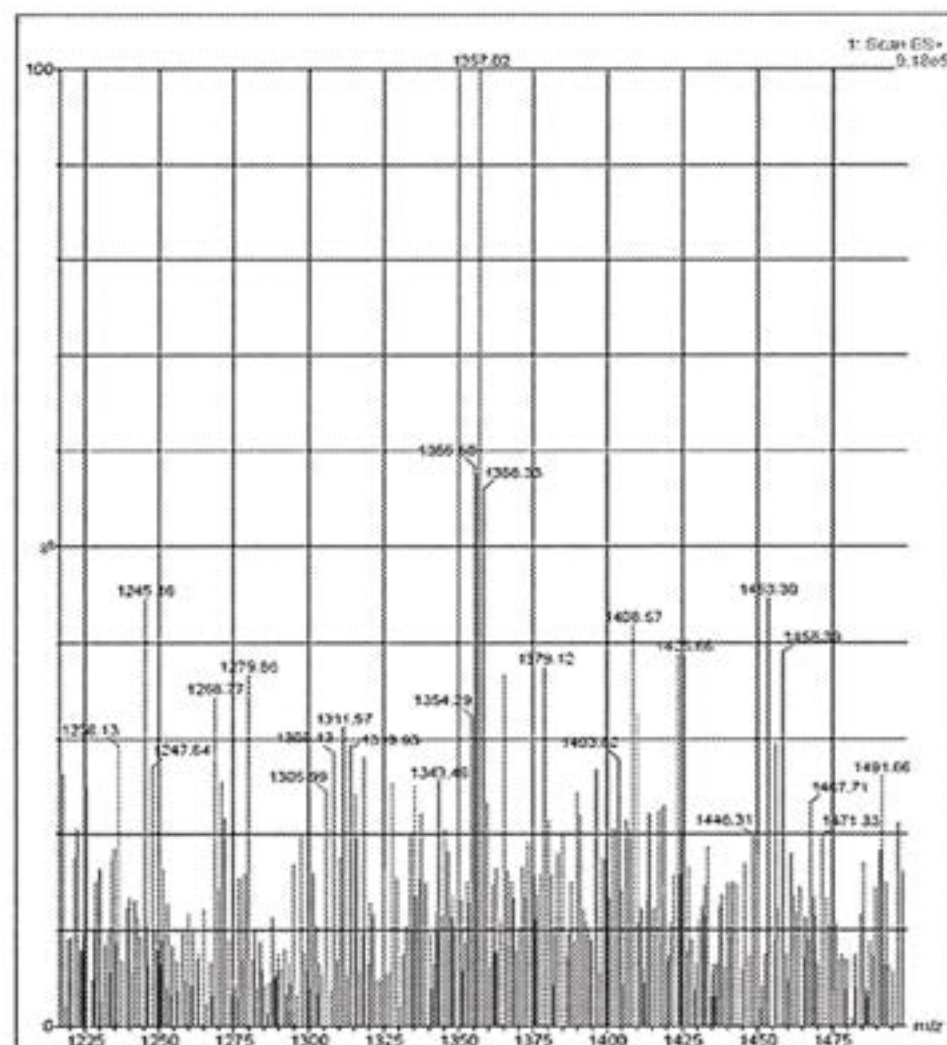


FIG. 12



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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only	
PCT/US24/23590 International Application No.	
08 April 2024 (08.04.2024) International Filing Date	
RO/US Name of receiving Office and "PCT International Application"	
Applicant's or agent's file reference (if desired) (25 characters maximum)	

Box No. I TITLE OF INVENTION	
METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
Rock BioMedical Inc. Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)	
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State (that is, country) of nationality: TW	
State (that is, country) of residence: TW	
This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
Wade Lin Prosyla Group PC 530 Technology Dr., Suite 100, Irvine, CA 92618, United States of America	
E-mail address* lpdocketing@prosylogroup.com	
Telephone No. 949-352-4590	
Facsimile No. 949-506-3690	
Agent's registration No. with the Office L1383	
* E-mail authorization: Indicating an e-mail address above authorizes the receiving Office, the International Searching Authority and the International Bureau, if they provide such a service, to send notifications exclusively by e-mail to that address, unless the following box is marked: ☐ notifications are requested to be sent exclusively by postal mail.	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) Chi-Huey WONG P.O. Box 8154 Rancho Santa Fe, CA 92067 United States of America	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) J eng Shin LEE 9 Bedford Lane Lincoln, MA 01773 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) Chen-Yo FAN Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: TW	State (that is, country) of residence: TW
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) Szu-Wen WANG Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only (If this check-box is marked, do not fill in below.) Applicant's registration No. with the Office
State (that is, country) of nationality: TW	State (that is, country) of residence: TW
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

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Supplemental Box

If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:
 - (i) **if more than one person is to be indicated as applicant and/or inventor** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
 - (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
 - (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
 - (iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
 - (v) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.
2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such case, write the name or two-letter code of each designated State concerned and the indication **"patent of addition," "certificate of addition," "inventor's certificate of addition"** or **"utility certificate of addition,"** the number of the parent application or parent patent or other parent grant and the date of grant of the parent patent or other parent grant or the date of filing of the parent application (Rules 4.11(a)(i) and 49bis.1(a) or (b)).
3. If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such case, write "United States of America" or "US" and the indication **"continuation"** or **"continuation-in-part"** and the number and the filing date of the parent application (Rules 4.11(a)(ii) and 49bis.1(d)).

Continuation of Box III:

Inventor No. 5:
Chung-Yi WU, Inventor Only
RM. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)
Nationality: TW
Residence: TW

Continuation of Box VI: Priority Claim

Priority Date: 08/04/2024
Application No.: TBD
Title: METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING VACCINES
National Appln Country: WO
Access Code: TBD

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s)
The International Bureau is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s).

Box No. V DESIGNATIONS				
The filing of this request constitutes under Rule 4.9(a) the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents. However, ☐ DE Germany is not designated for any kind of national protection ☐ JP Japan is not designated for any kind of national protection ☐ KR Republic of Korea is not designated for any kind of national protection <i>(The check-boxes above may only be used to exclude (irrevocably) the designations concerned if, at the time of filing or subsequently under Rule 26bis.1, the international application contains in Box No. VI a priority claim to an earlier national application filed in the particular State concerned, in order to avoid the ceasing of the effect, under the national law, of this earlier national application.)</i>				
Box No. VI PRIORITY CLAIM AND DOCUMENT				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 08/04/2023	63458102	US		
item (2) 02/10/2023	63587231	US		
item (3) 05/04/2024	63575093	US		
☒ Further priority claims are indicated in the Supplemental Box.				
Furnishing the priority document(s):				
☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application(s) was filed with the receiving Office which, for the purposes of this international application, is the receiving Office) identified above as: ☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box				
☒ The International Bureau is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s) indicated below (if the earlier application(s) is available to it from a digital library): ☒ item (1) access code 8787 ☒ item (2) access code 2122 ☒ item (3) access code 2760 ☐ other, see Supplemental Box				
Restore the right of priority: the receiving Office is requested to restore the right of priority for the earlier application(s) identified above or in the Supplemental Box as item(s) (). (See also the Notes to Box No. VI; further information must be provided to support a request to restore the right of priority.)				
Incorporation by reference: where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a), or an element or part of the description, claims or drawings referred to in Rule 20.5bis(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.				
Box No. VII INTERNATIONAL SEARCHING AUTHORITY				
Choice of International Searching Authority (ISA) (if more than one International Searching Authority is competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used): ISA/ <u>US</u>				

Box No. IX CHECK LIST for electronic filing with RO/US – only to be used when filing via the USPTO electronic filing system			
This international application contains the following:	Number of sheets	This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item):	Number of items
(a) request form PCT/RO/101 (including any declarations and supplemental sheets)	5	1. ☒ fee calculation sheet	1
(b) description	78	2. ☐ original separate power of attorney	
(c) claims	8	3. ☐ original general power of attorney	
(d) abstract	1	4. ☐ copy of general power of attorney; reference number:	
(e) drawings (if any)	10	5. ☐ priority document(s) identified in Box No. VI as item(s)	
Total number of sheets	102	6. ☐ Translation of international application into (language):	
(f) sequence listing part of the description		7. ☐ separate indications concerning deposited microorganism or other biological material	
☐ filed as a WIPO Standard ST.26 XML file		8. ☐ copy of results of earlier search(es) (Rule 12bis.1(a))	
☐ WILL BE filed separately on physical data carrier(s) as a WIPO Standard ST.26 XML file , on the same day		9. ☐ other (specify):	
Indicate type and number of physical data carrier(s)			
Figure of the drawings which should accompany the abstract:	5	Language of filing of the international application:	
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE			
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).			
/Wade Lin/ Wade Lin, Reg. No. L1383			

For receiving Office use only			
1. Date of actual receipt of the purported international application:	08 April 2024 (08.04.2024)		2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:			
4. Date of timely receipt of the required corrections under PCT Article 11(2):			
5. International Searching Authority (if two or more are competent):	US	6. ☐ Transmittal of search copy delayed until search fee is paid	

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

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753

This sheet is not part of and does not count as a sheet of the international application.

PCT
FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

PCT/US24/23590

International Application No.

08 April 2024 (08.04.2024)

Date stamp of the receiving Office

Applicant's or agent's file reference

Applicant

CALCULATION OF PRESCRIBED FEES

(Applicants may be entitled to a reduction of certain fees as indicated in the PCT Fee Tables
(www.wipo.int/pct/en/fees.pdf))

1. TRANSMITTAL FEE: 104.00 [T]

2. SEARCH FEE: 872.00 [S]

International search to be carried out by: US

3. INTERNATIONAL FILING FEE

Enter total number of sheets indicated in Box No IX: 102

[i1] Fixed amount for the first 30 sheets 1445.00 [i1]

[i2] 72 x 18.00 = 1296.00 [i2]
number of sheets fee per sheet
in excess of 30

Add amounts entered at i1 and i2 and enter total at I: 2741.00 [I]

(Applicants from certain States are entitled to a reduction of 90% of the international filing fee (see
www.wipo.int/pct/en/fees/fee_reduction.pdf). Where the applicant is (or all applicants are) so entitled, the total
to be entered at I is 10% of the international filing fee.)

4. FEE FOR PRIORITY DOCUMENT (if applicable): [P]

5. FEE FOR RESTORATION OF THE RIGHT OF PRIORITY (if
applicable): [RP]

6. FEE FOR EARLIER SEARCH DOCUMENTS (if applicable): [ES]

7. TOTAL FEES PAYABLE 3717.00

Add amounts entered at T, S, I, P, RP and ES,
and enter total in the TOTAL box

TOTAL

MODE OF PAYMENT (Not all modes of payment may be available at all receiving Offices)

- ☐ credit card (details should not be included on this sheet) ☒ authorization to charge deposit or current account (see below) ☐ bank transfer ☐ cash
☐ postal money order ☐ check ☐ revenue stamps ☐ other (specify):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT OR CURRENT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

- ☒ Authorization to charge the total fees indicated above.
☒ (This check-box may be marked only if the conditions for deposit or current accounts of the receiving Office so permit) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.
☐ Authorization to charge the fee for priority document.

Receiving Office: RO/ US

Deposit or Current Account No.: 603395

Date: April 8, 2024

Name: Wade Lin

Signature: /Wade Lin/

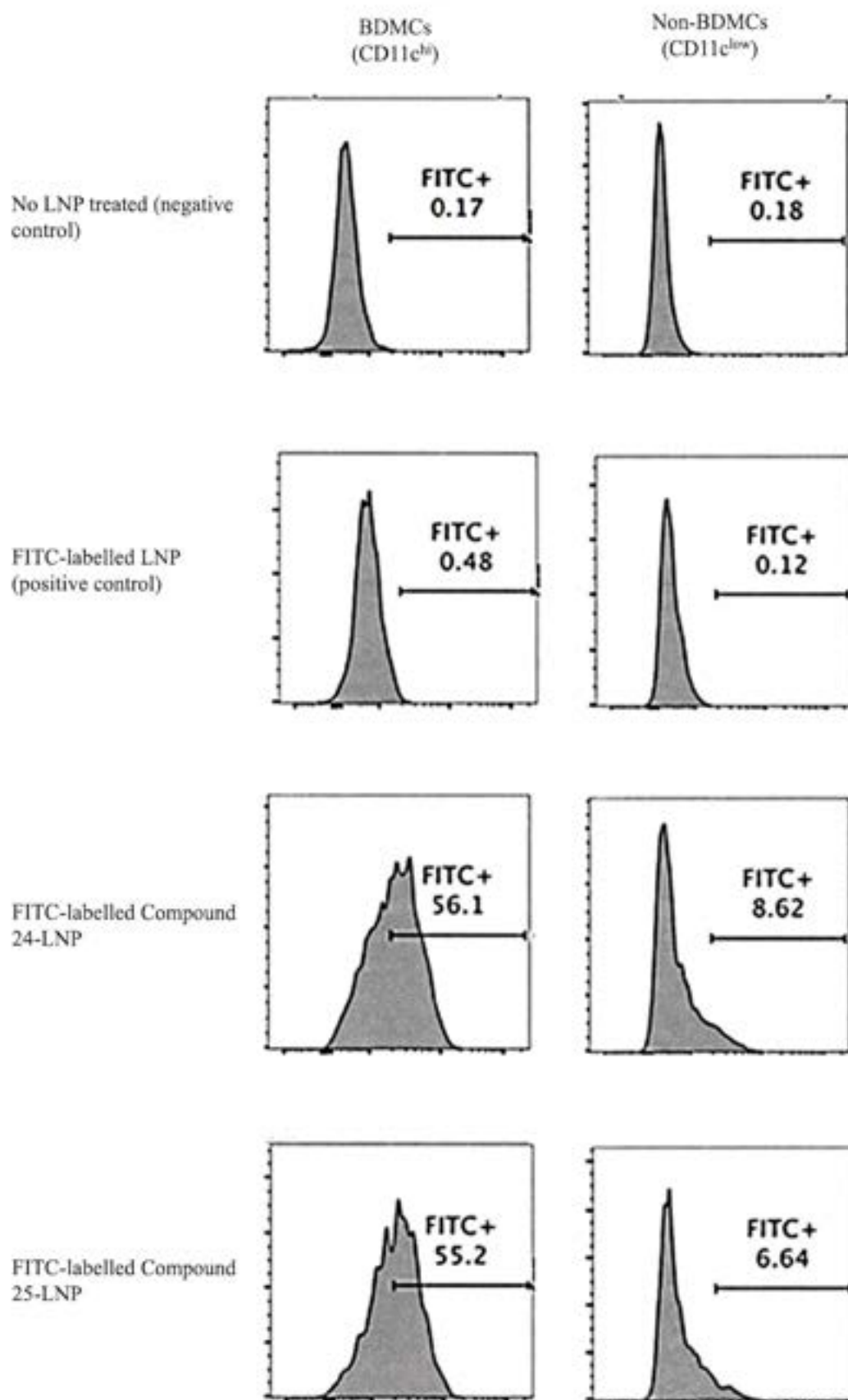


FIG. 1

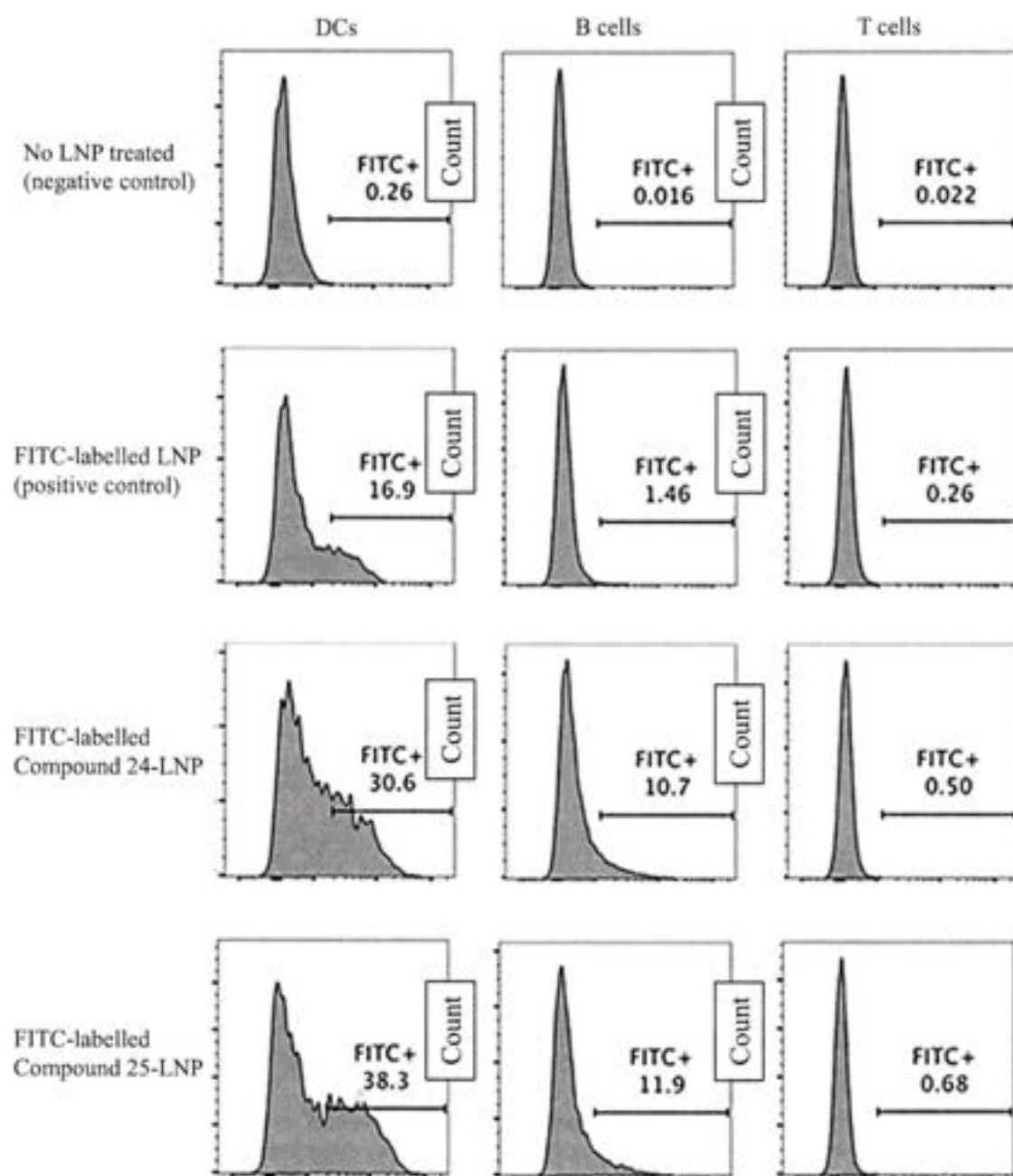


FIG. 2

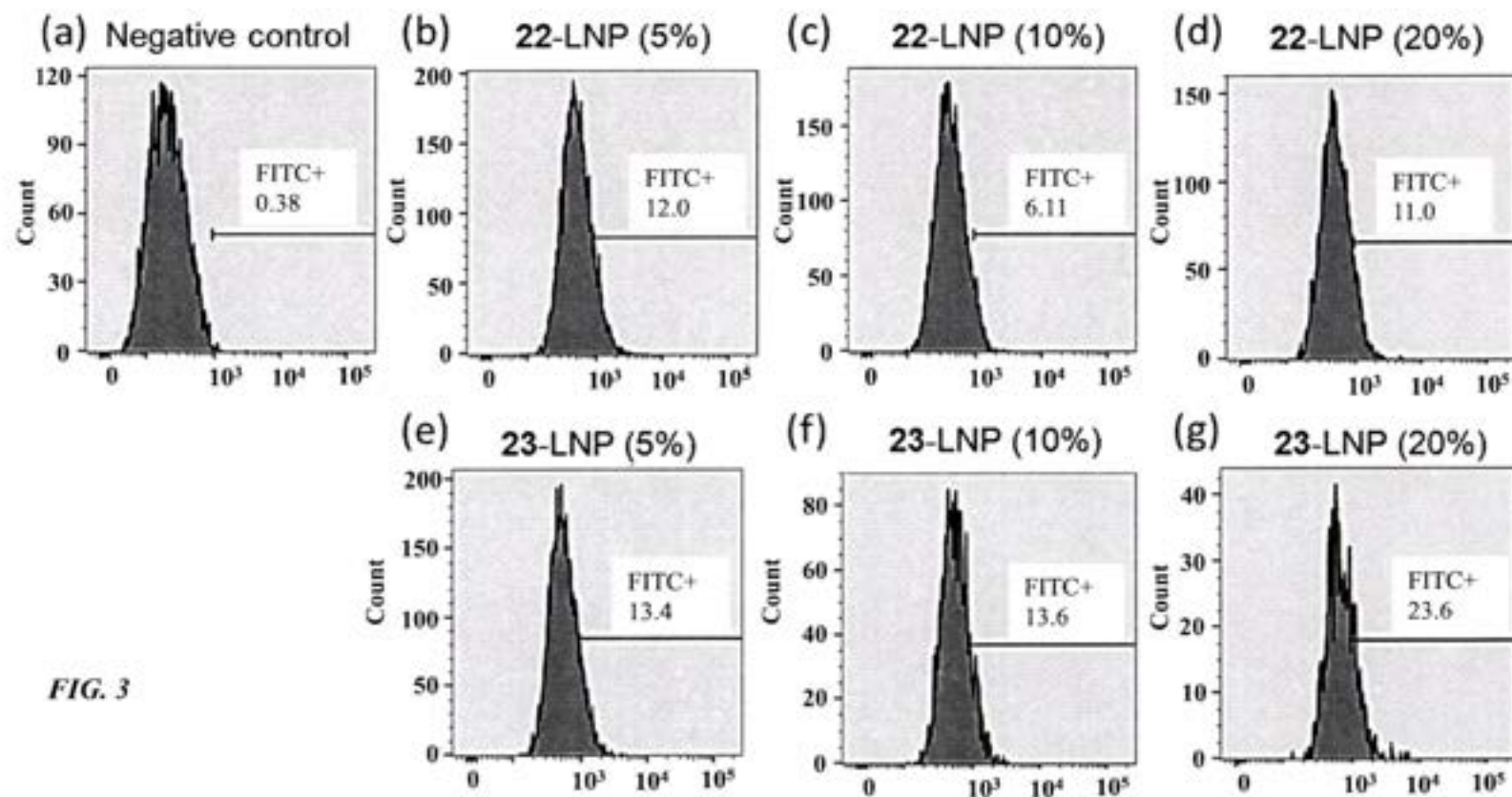


FIG. 3

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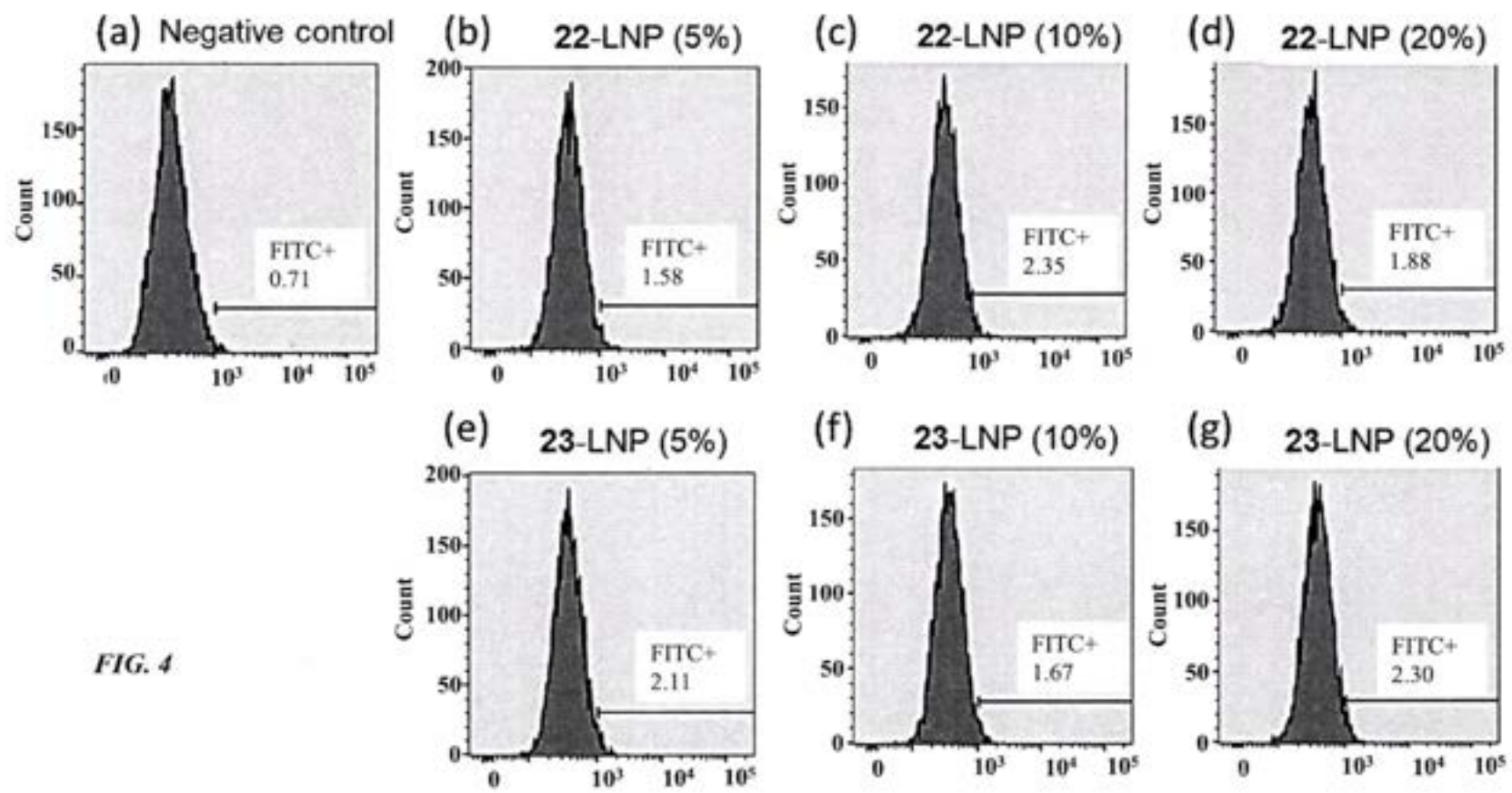


FIG. 4

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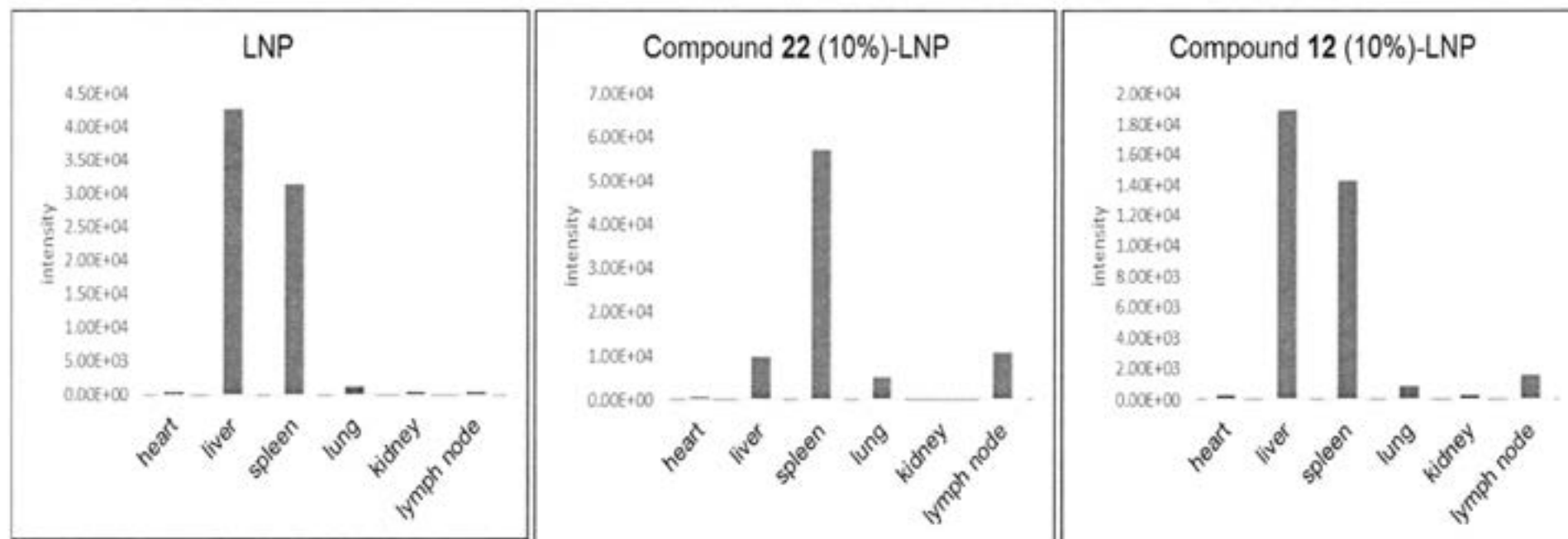


FIG. 5

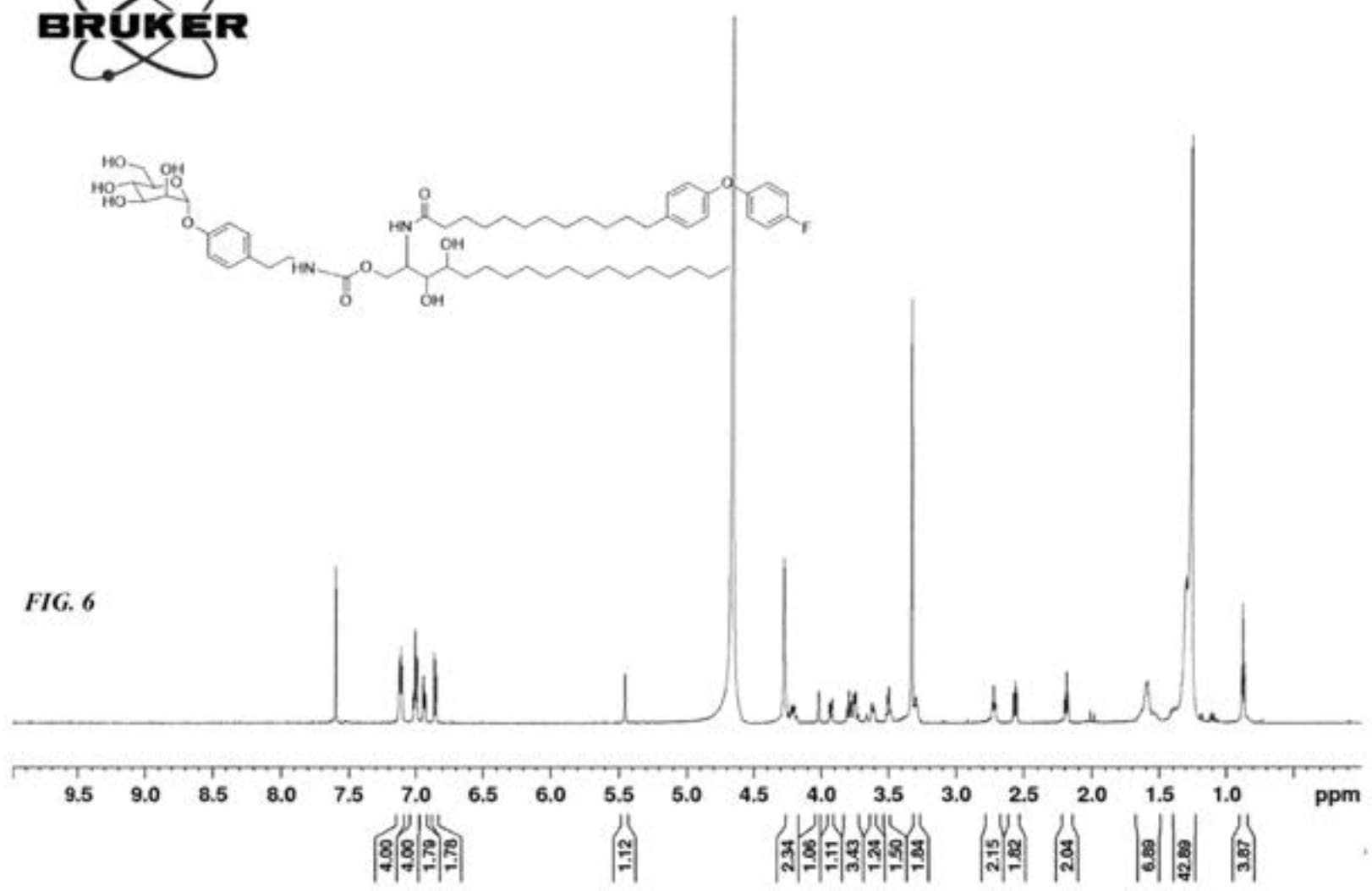


FIG. 6

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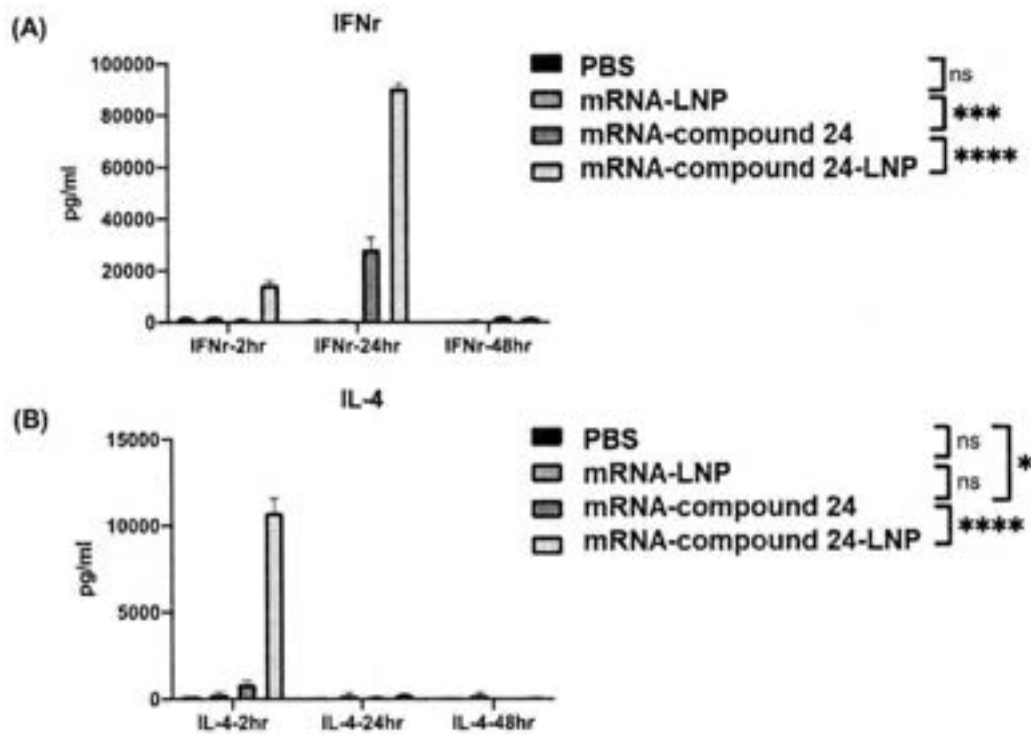


FIG. 8

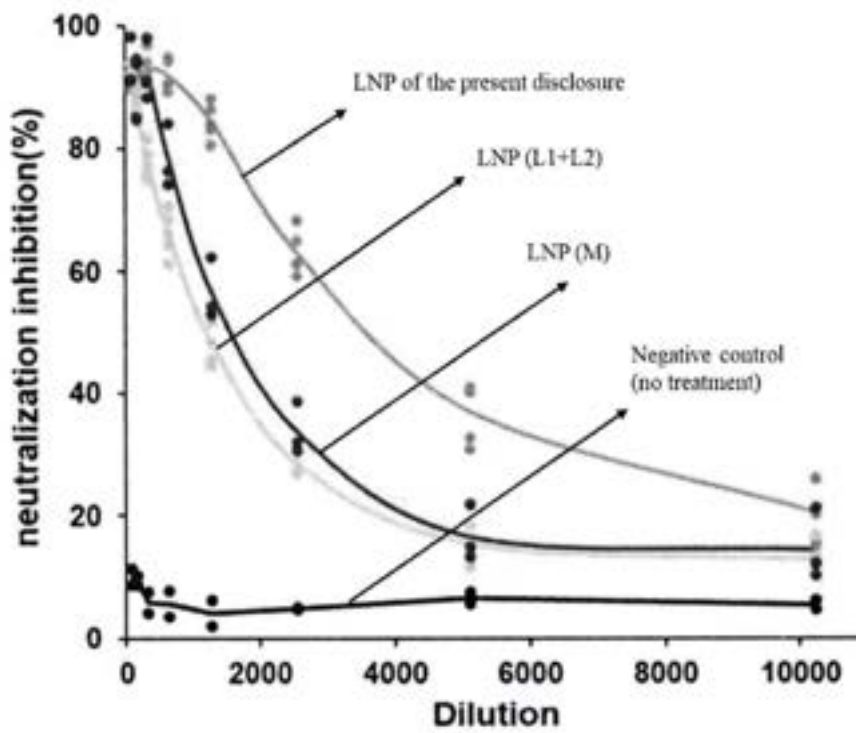


FIG. 9

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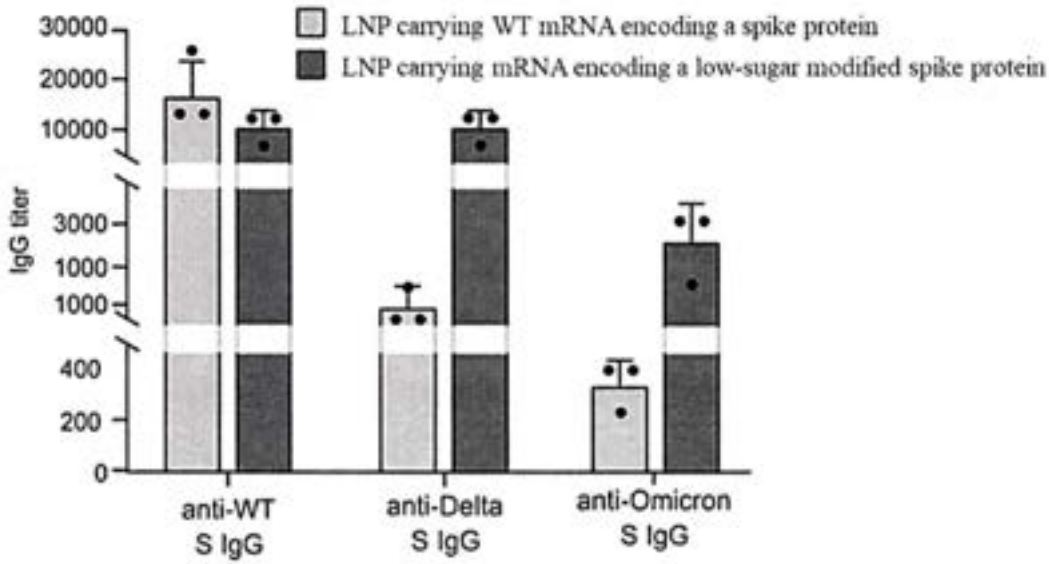


FIG. 10

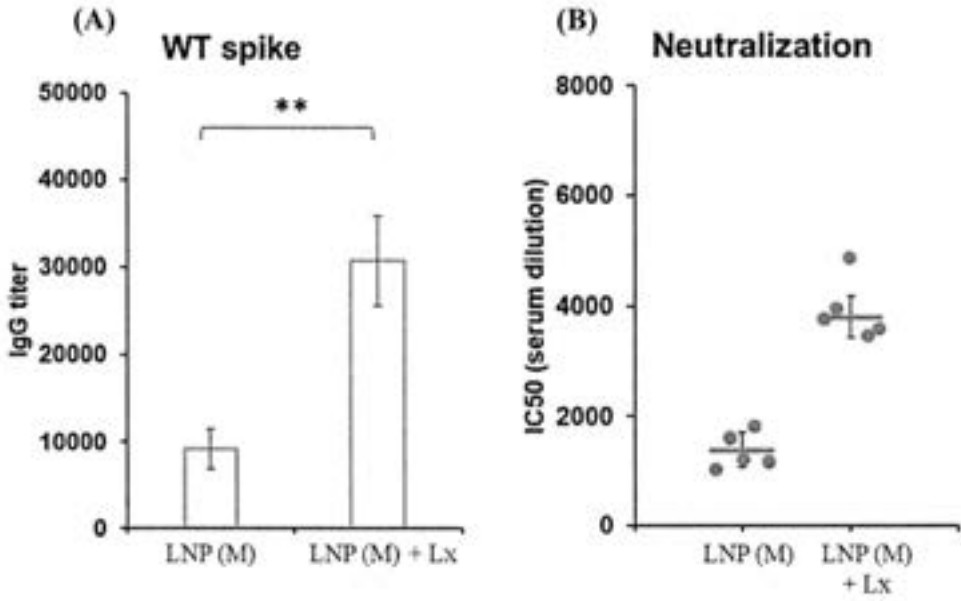


FIG. 11

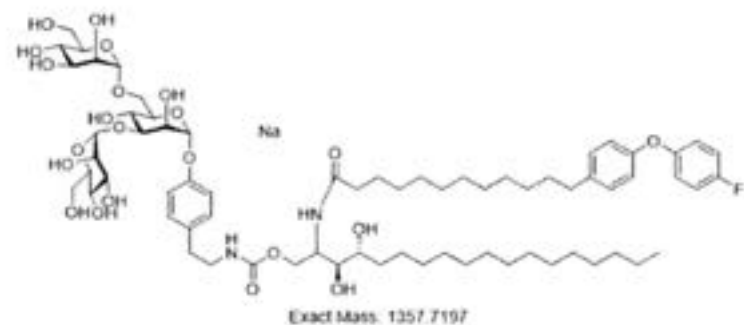
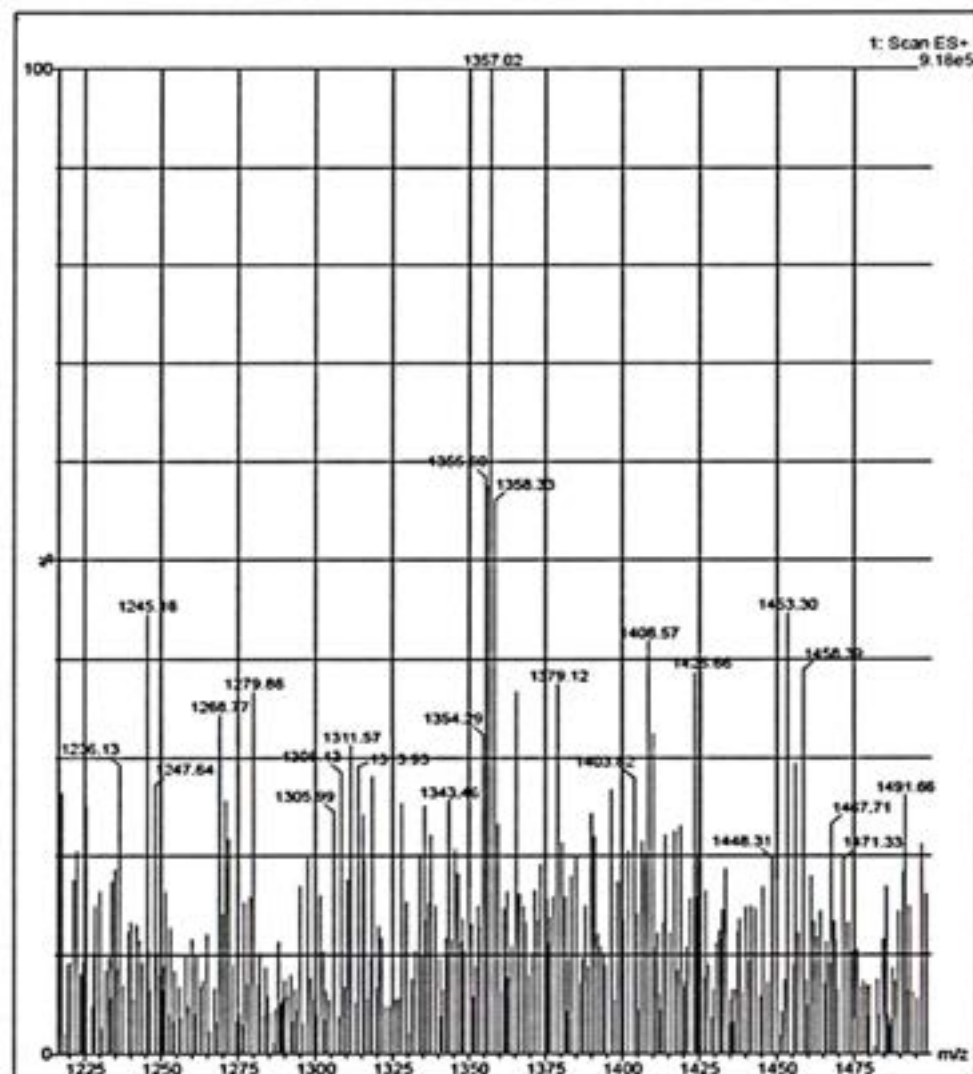


FIG. 12



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**INTERNATIONAL BUREAU OF WIPO
UNDER THE PATENT COOPERATION TREATY**

In re the PCT Application of	)	Conf. No. 2227
	)	
ROCK BIOMEDICAL, INC.	)	Cust. No. 169053
	)	
International Application No.: PCT/US24/23597	)	
	)	
Filed: April 8, 2024	)	
	)	
For: METHODS AND COMPOSITIONS FOR	)	
DENDRITIC CELL TARGETING VACCINES	)	
	)	
	)	

REQUEST FOR CHANGE OF APPLICANT ADDRESS UNDER RULE 92bis.1

The International Bureau of WIPO
PCT Receiving Office Section
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Dear Sir/Madam:

Pursuant to Rule PCT 92bis, Applicant hereby requests a change of applicant. Please change the Applicant from "ROCK BIOMEDICAL, INC. and Jeng Shin LEE" to "ROCK BIOMEDICAL, INC." only. Jeng Shin LEE should now be listed as an inventor only. Please correct Applicant information accordingly.

765

We respectfully request issuance of a Recordation of Change Form PCT/IB/306 and notification to the respective offices accordingly.

Respectfully submitted,

Dated: September 8, 2025

By : /Kay Yang/
Kay Yang
Patent Agent
Reg. No. 61072

PROSYLA GROUP, PC
530 Technology Dr., Suite 100
Irvine, CA 92618
(O) (949) 352-4590
(F) (949) 506-3690
Customer No. 169053

Pending processing

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (25 characters maximum)

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Box No. I TITLE OF INVENTION

METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING VACCINES

Box No. II APPLICANT

☐ This person is also inventor

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

Rock BioMedical Inc.
Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd.,
Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)

E-mail address*

Telephone No.

Facsimile No.

Applicant's registration No. with the Office

* E-mail authorization: Indicating an e-mail address above authorizes the receiving Office, the International Searching Authority and the International Bureau, if they provide such a service, to send notifications exclusively by e-mail to that address, unless the following box is marked:

☐ notifications are requested to be sent exclusively by postal mail.

State (that is, country) of nationality:

TW

State (that is, country) of residence:

TW

This person is applicant for the purposes of:

☒ all designated States☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Wade Lin
Prosyla Group PC
530 Technology Dr., Suite 100, Irvine, CA 92618, United
States of America

E-mail address*

ipdocketing@prosytagroup.com

Telephone No.

949-352-4590

Facsimile No.

949-506-3690

Agent's registration No. with the Office

L1383

* E-mail authorization: Indicating an e-mail address above authorizes the receiving Office, the International Searching Authority and the International Bureau, if they provide such a service, to send notifications exclusively by e-mail to that address, unless the following box is marked:

☐ notifications are requested to be sent exclusively by postal mail.

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

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Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) <i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> Chi-Huey WONG P.O. Box 8154 Rancho Santa Fe, CA 92067 United States of America	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality: US	State <i>(that is, country)</i> of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> Jeng Shin LEE 9 Bedford Lane Lincoln, MA 01773 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality: US	State <i>(that is, country)</i> of residence: US
This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> Chen-Yo FAN Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality: TW	State <i>(that is, country)</i> of residence: TW
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> Szu-Wen WANG Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality: TW	State <i>(that is, country)</i> of residence: TW
This person is applicant for the purposes of: ☐ all designated States ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Supplemental Box

If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than one person is to be indicated as applicant and/or inventor and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such case, write the name or two-letter code of each designated State concerned and the indication "patent of addition," "certificate of addition," "inventor's certificate of addition" or "utility certificate of addition," the number of the parent application or parent patent or other parent grant and the date of grant of the parent patent or other parent grant or the date of filing of the parent application (Rules 4.11(a) (i) and 49bis.1(a) or (b)).

3. If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such case, write "United States of America" or "US" and the indication "continuation" or "continuation-in-part" and the number and the filing date of the parent application (Rules 4.11(a)(ii) and 49bis.1(d)).

Continuation of Box III:

inventor No. 5:

Chung-Yi WU, Inventor Only

RM. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202, Taiwan (R.O.C.)

Nationality: TW

Residence: TW

Continuation of Box VI:

Filing Date: 02/02/2024

Application No.: 63549343

National Country: US

Access Code: 7068

Filing Date: 05/04/2024

Application No.: 63575093

National Country: US

Access Code: 2760

Filing Date: 08/04/2024

Application No.: TBD

Title: METHODS AND COMPOSITIONS FOR DENDRITIC CELL TARGETING NANO-DELIVERY

National Country: WO

Access Code: TBD

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s)

The International Bureau is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s)

Box No. V DESIGNATIONS

The filing of this request **constitutes under Rule 4.9(a) the designation** of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany **is not designated** for any kind of national protection
- ☐ JP Japan **is not designated** for any kind of national protection
- ☐ KR Republic of Korea **is not designated** for any kind of national protection

(The check-boxes above may only be used to exclude (irrevocably) the designations concerned if, at the time of filing or subsequently under Rule 26bis.1, the international application contains in Box No. VI a priority claim to an earlier national application filed in the particular State concerned, in order to avoid the ceasing of the effect, under the national law, of this earlier national application.)

Box No. VI PRIORITY CLAIM AND DOCUMENT

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 08/04/2023	63458102	US		
item (2) 02/10/2023	63587231	US		
item (3) 09/10/2023	63588932	US		

☒ Further priority claims are indicated in the Supplemental Box.

Furnishing the priority document(s):

☒ The **receiving Office** is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application(s) was filed with the receiving Office which, for the purposes of this international application, is the receiving Office) identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

☒ The **International Bureau** is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s) indicated below (if the earlier application(s) is available to it from a digital library):

☒ item (1) access code 8787 ☒ item (2) access code 2122 ☒ item (3) access code 6852 ☒ other, see Supplemental Box

Restore the right of priority: the receiving Office is requested to restore the right of priority for the earlier application(s) identified above or in the Supplemental Box as item(s) (_____). (See also the Notes to Box No. VI: further information **must** be provided to support a request to restore the right of priority.)

Incorporation by reference: where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a), or an element or part of the description, claims or drawings referred to in Rule 20.5bis(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if more than one International Searching Authority is competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA/ US

787
770**Box No. IX CHECK LIST for electronic filing with RO/US – only to be used when filing via the USPTO electronic filing system**

This international application contains the following:	Number of sheets	This international application is accompanied by the following item(s) (<i>mark the applicable check-boxes below and indicate in right column the number of each item</i>):	Number of items
(a) request form PCT/RO/101 (including any declarations and supplemental sheets)	5	1. ☒ fee calculation sheet.....	1
(b) description	132	2. ☐ original separate power of attorney	:
(c) claims	10	3. ☐ original general power of attorney.....	:
(d) abstract	1	4. ☐ copy of general power of attorney; reference number:	:
(e) drawings (if any).....	10	5. ☐ priority document(s) identified in Box No. VI as item(s)	:
Total number of sheets	158	6. ☐ Translation of international application into (language):	:
(f) sequence listing part of the description		7. ☐ separate indications concerning deposited microorganism or other biological material	:
☒ filed as a WIPO Standard ST.26 XML file		8. ☐ copy of results of earlier search(es) (Rule 12bis.1(a)) ...	:
☐ WILL BE filed separately on physical data carrier(s) as a WIPO Standard ST.26 XML file , on the same day		9. ☐ other (<i>specify</i>):	:
Indicate type and number of physical data carrier(s)			
Figure of the drawings which should accompany the abstract:		Language of filing of the international application:	

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

/Wade Lin/
Wade Lin, Reg. No. L1383**For receiving Office use only**

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent):	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PCT**FEE CALCULATION SHEET****Annex to the Request**

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference

Applicant

CALCULATION OF PRESCRIBED FEES*(Applicants may be entitled to a reduction of certain fees as indicated in the PCT Fee Tables (www.wipo.int/pct/en/fees.pdf))*1. TRANSMITTAL FEE: 104.00 **T**2. SEARCH FEE: 872.00 **S**International search to be carried out by: US

3. INTERNATIONAL FILING FEE

Enter total number of sheets indicated in Box No IX: 158**i1** Fixed amount for the first 30 sheets 1445.00 **i1****i2** 128 x 18.00 = 2304.00 **i2**
number of sheets fee per sheet
in excess of 30Add amounts entered at i1 and i2 and enter total at I: 3749.00 **I***(Applicants from certain States are entitled to a reduction of 90% of the international filing fee (see www.wipo.int/pct/en/fees/fee_reduction.pdf). Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 10% of the international filing fee.)*4. FEE FOR PRIORITY DOCUMENT (if applicable): **P**5. FEE FOR RESTORATION OF THE RIGHT OF PRIORITY (if applicable): **RP**6. FEE FOR EARLIER SEARCH DOCUMENTS (if applicable): **ES**

7. TOTAL FEES PAYABLE 4725.00

Add amounts entered at T, S, I, P, RP and ES,
and enter total in the TOTAL box**TOTAL****MODE OF PAYMENT** (Not all modes of payment may be available at all receiving Offices)

- ☐ credit card (details should not be included on this sheet) ☒ authorization to charge deposit or current account (see below) ☐ bank transfer ☐ cash
- ☐ postal money order ☐ check ☐ revenue stamps ☐ other (specify):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT OR CURRENT ACCOUNT*(This mode of payment may not be available at all receiving Offices)*

- ☒ Authorization to charge the total fees indicated above.
- ☒ (This check-box may be marked only if the conditions for deposit or current accounts of the receiving Office so permit) Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.
- ☐ Authorization to charge the fee for priority document.

Receiving Office: RO/ USDeposit or Current Account No.: 603395Date: April 8, 2024Name: Wade LinSignature: /Wade Lin/

800
772

UNITED STATES PATENT AND TRADEMARK OFFICE AS RECEIVING OFFICE
UNDER THE PATENT COOPERATION TREATY

In re Patent Application of
Rock BioMedical Inc.
Serial No.: PCT/US24/23597

Filing Date: April 8, 2024

For: METHODS AND COMPOSITIONS
FOR DENDRITIC CELL TARGETING
VACCINES

Customer No.: 169053

Confirmation No.: 2227

Group Art Unit: n/a

Authorized Officer: Darlene Proctor

**RESPONSE TO INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL
APPLICATION**

VIA Patent Center

Mail Stop PCT

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Dear Commissioner:

In response to the Invitation to Correct Defects in the International Application, dated May 2, 2024, to which the deadline for responding is July 2, 2024, Applicant submits the following Amendments and Remarks.

An **Amendment to the Specification** begins on page **2** of this Response.

An **Amendment to the Drawings** begins on page **3** of this Response.

Remarks begin on page **4** of this Response.

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AMENDMENTS TO THE SPECIFICATION

Applicant provides a Substitute Specification in a clean format (total 146 pages). The amendment to the specification adjusts the line spacing of the specification, claims, and abstract to 1½ and ensures the text matter on the sheets in dark, indelible color. No new matter is added to the Specification with these amendments.

AMENDMENTS TO THE DRAWINGS

The Applicant provides a drawing replacement sheet (total 10 sheets) in response to Invitation to Correct Defects in the International Application. No new matter is introduced.

REMARKS

The Invitation to Correct Defects in the International Application invites the Applicant to address the defects in the text matter and drawings of the international application. Accordingly, the Applicant submits a Substitute Specification in which the line spacing of the specification, claims, and abstract is adjusted to 1 1/2 and the text matter on the sheets is in dark, indelible color. The Applicant also submits a drawing replacement sheet. In the drawing replacement sheet, blurry texts are made clear and enlarged if necessary. No new matter is introduced.

CONCLUSION

Applicant respectfully the amended specification is in compliance with PCT practice and format. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below. In the event the U.S. Patent and Trademark Office determines that an extension and/or other relief is required, applicant petitions for any required relief authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document throughout the pendency of the application to Deposit Account No. 60-3395 referencing docket no. A1000-01100PCT-3.

Respectfully submitted,

Dated: July 1, 2024

By: /Wade Lin/
Wade Lin
Registration No. 61,072

PROSYLA GROUP PC
Customer No. 169053
530 Technology Drive, Suite 100
Irvine, CA 92618
Telephone: (949) 352-4590
Facsimile: (949) 506-3690

809
776

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION,
OBTENTION OR TRANSMITTAL
OF PRIORITY DOCUMENT
(PCT Administrative Instructions, Section 411)

To:

LIN, Wade
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 13 June 2024 (13.06.2024)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference A1000-01100PCT-3	
International application No. PCT/US2024/023597	International filing date (day/month/year) 08 April 2024 (08.04.2024)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 08 April 2023 (08.04.2023)
Applicant ROCK BIOMEDICAL INC. et al	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis). This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
08 April 2023 (08.04.2023)	63/458.102	US	07 May 2024 (07.05.2024)
02 October 2023 (02.10.2023)	63/587.231	US	07 May 2024 (07.05.2024)
09 October 2023 (09.10.2023)	63/588.932	US	07 May 2024 (07.05.2024)
02 February 2024 (02.02.2024)	63/549.343	US	07 May 2024 (07.05.2024)
05 April 2024 (05.04.2024)	63/575.093	US	13 May 2024 (13.05.2024)
08 April 2024 (08.04.2024)	PCT/US24/23590	US	12 June 2024 (12.06.2024)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-bis) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-bis)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-bis), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Sun Hwa Lee e-mail pct.team1@wipo.int Telephone No. +41 22 338 74 01
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From the INTERNATIONAL BUREAU

PCT

SECOND AND SUPPLEMENTARY NOTICE
INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES
WHICH APPLY THE 30 MONTH TIME
LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

LIN, Wade
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)

14 August 2025 (14.08.2025)

Applicant's or agent's file reference

A1000-01100PCT-3

IMPORTANT NOTICE

International application No.

PCT/US2024/023597

International filing date (day/month/year)

08 April 2024 (08.04.2024)

Priority date (day/month/year)

08 April 2023 (08.04.2023)

Applicant

ROCK BIOMEDICAL INC. et al

1. **ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does not apply**, please see Form PCT/IB/308(First Notice) issued previously.

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
17 October 2024 (17.10.2024)

EP

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1:

AE, AG, AL, AM, AO, AP, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OA, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. **TIME LIMITS** for entry into the national phase

For the designated or elected Office(s) listed above, the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **30 MONTHS** from the priority date.

In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For **regular updates on the applicable time limits** (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/guide/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Sun Hwa Lee

e-mail pct.team1@wipo.int

Telephone No. +41 22 338 74 01

From the INTERNATIONAL BUREAU

PCT

FIRST NOTICE INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES WHICH
DO NOT APPLY THE 30 MONTH TIME LIMIT
UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

LIN, Wade
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 14 November 2024 (14.11.2024)		
Applicant's or agent's file reference A1000-01100PCT-3		IMPORTANT NOTICE
International application No. PCT/US2024/023597	International filing date (day/month/year) 08 April 2024 (08.04.2024)	Priority date (day/month/year) 08 April 2023 (08.04.2023)
Applicant ROCK BIOMEDICAL INC. et al		

ATTENTION: For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does apply**, please see Form PCT/IB/308(Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
17 October 2024 (17.10.2024)

None

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1:

LU, TZ

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

TIME LIMITS for entry into the national phase

For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of **19 months** from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **20 MONTHS** from the priority date.

In practice, **time limits other than the 20-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For **regular updates on the applicable time limits** (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/guide/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Sun Hwa Lee e-mail pct.team1@wipo.int Telephone No. +41 22 338 74 01
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		Fecha: 2024-10-29

Folio: 795

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Dependencia Productora: Dir. De Signos Distintivos		Código: 2010
Tipo de Radicación: Entrada: ☒ Salida: ☐ Traslado: ☐ Convenio: ☐		
Radicador:		
DESCRIPCIÓN DE LA PRUEBA		
Nombre de la Serie / Subserie:		Código:
Expediente: NC2025/0015487	Solicitante / Quejoso / Demandante / otros.	
Prueba excluida: USB		
N°. de Pruebas excluidas: 1		
Fecha de emisión de la Prueba:		
UBICACIÓN DE LA PRUEBA EN LA SECCIÓN DE MATERIAL GRÁFICO		
N°. de bloque:		Otras especificaciones:
N°. de rodante:		
N°. de estante:		
N°. de archivador:		
N°. de entropaño:		
N°. de gaveta:		
N°. de caja:		
IMPORTANTE: - El material gráfico o especial que se encuentra dentro o anexo a las unidades documentales o expedientes, debe extraerse y llevarse a aquella sección del archivo que se haya dispuesto para conservar documentos en distintos formatos. - Entiéndase por material gráfico todos aquellos dibujos, croquis, mapas, planos, fotografías, ilustraciones, pictografías, códices, prensa, objetos tridimensionales, entre otros.		
Observaciones: SE ANEXA 1 USB EN BUEN ESTADO QUE CONTIENE : 1 APLICACIÓN, 1 DOCUMENTO DE MICROSOFT WORD Y 1 MICROSOFT EDGE HTML DOCUMENT. 		Unidad de Conservación ☐ Bolsa ☐ Caja ☐ Libro ☐ Sobre ☐ Tomo ☐ Otro Soporte Documental ☐ CD ☐ Disco duro ☐ DVD ☐ Folleto ☐ Periódico ☐ Plano ☐ Publicación Periódica ☒ USB ☐ Otro: _____

Nota: En el ítem de "Observaciones", se describe las características físicas de o las pruebas, como el nombre de la marca, color, contenido, estado, (o si no tiene contenido, o si está en mal estado); en el caso de los medios ópticos y magnéticos describir las propiedades y colocar el peso de la información (ejemplo: 131 GB).