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(54) Title: LIPID NANOPARTICLES COMPRISING STEROL-MODIFIED PHOSPHOLIPIDS

(57) Abstract: Provided is LNPs comprising phospholipids containing a sterol moiety. LNPs comprising such phospholipids have potential applications in mRNA vaccine technology. Provided are compositions comprising the LNPs and methods for using the LNPs or the compositions described above.

LIPID NANOPARTICLES COMPRISING STEROL-MODIFIED PHOSPHOLIPIDS

FIELD

The invention relates to lipid nanoparticles comprising phospholipids that contain a sterol moiety.

BACKGROUND

Lipid nanoparticles (or LNPs) are known vehicles for delivering biologically active agents to cells. LNPs have recently become particularly important in delivering nucleic acids to cells, due to issues with *in vivo* stability and cellular permeability of nucleic acids alone. One important application of nucleic acid-loaded LNPs are mRNA vaccines.

BRIEF SUMMARY

The present application provides phospholipids containing a sterol moiety that can be used to construct lipid nanoparticles. In one aspect, the lipid nanoparticles are useful for delivering nucleic acids, *e.g.* mRNA, to one or more cells. In one aspect, the application provides a method for expressing protein in a cell by delivering nucleic acids to the cell via the lipid nanoparticle comprising a phospholipid that contains a sterol moiety.

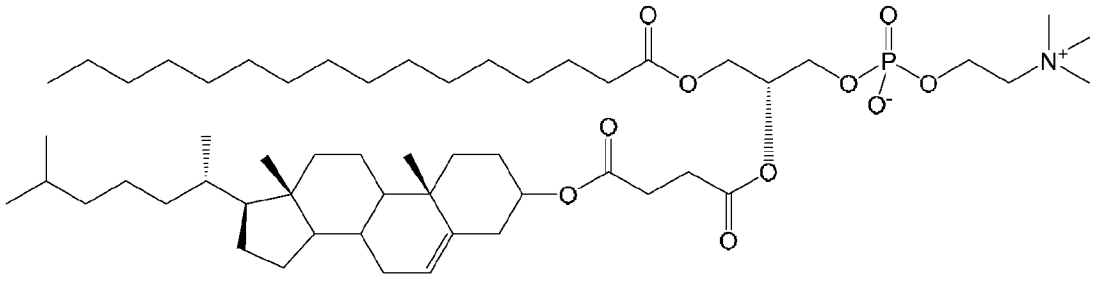
Also provided herein are lipid nanoparticles (LNPs) comprising

a phospholipid containing a sterol moiety;

an ionizable lipid; and

a polymer conjugated lipid.

In some embodiments, the phospholipid has a structure selected from:



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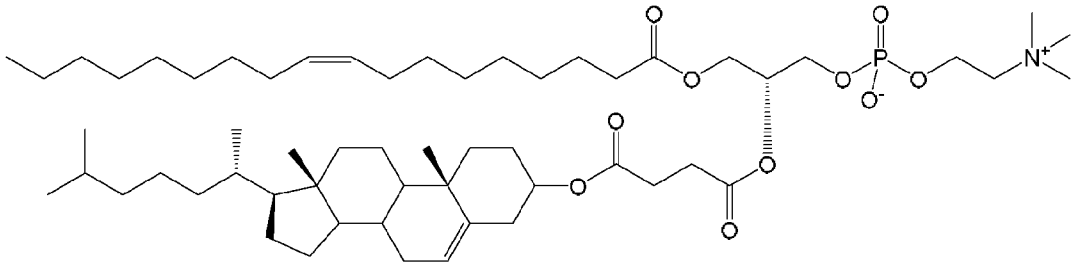

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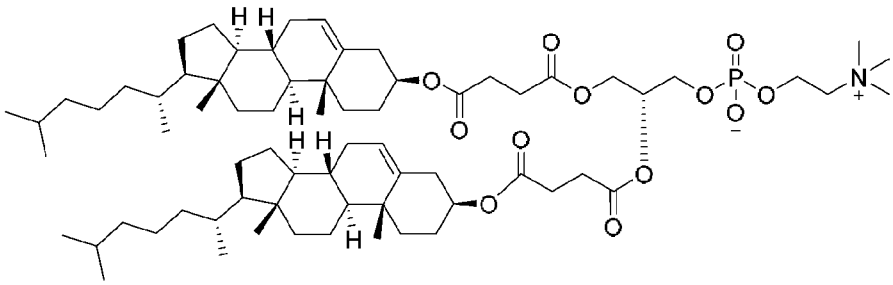
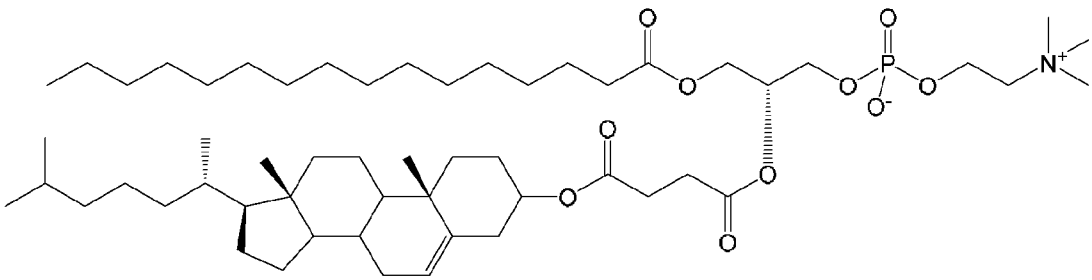
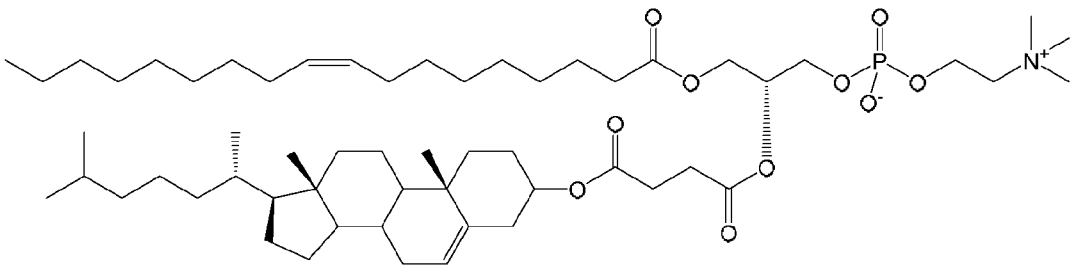


In some embodiments, the phospholipid has the structure:



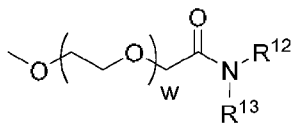
In some embodiments, the phospholipid has the structure:



In some embodiments, the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1. In some embodiments, the molar ratio of ionizable lipid to phospholipid is from 15:1 to 5:1. In some embodiments, the ionizable lipid comprises from 40 to 80 mol% of a total amount of lipids in the LNP. In some embodiments, the ionizable lipid comprises from 50 to 70 mol% of the total amount of lipids in the LNP. In some embodiments, the ionizable lipid is a

cationic lipid. In some embodiments, the ionizable lipid is a compound according to any one of the formula selected from 01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, or wherein the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5.

In some embodiments, the polymer conjugated lipid comprises from 0.5 to 5 mol% of the total amount of lipids in the LNP. In some embodiments, the polymer conjugated lipid comprises from 1 to 2 mol% of the total amount of lipids in the LNP. In some embodiments, the polymer conjugated lipid comprises 1.5 mol% of the total amount of lipids in the LNP. In some embodiments, the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid of from 1:2 to 1:20. In some embodiments, the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid of from 1:3 to 1:18. In some embodiments, the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid from 1:5 to 1:10. In some embodiments, the polymer conjugated lipid is a PEGylated lipid. In some embodiments, the polymer conjugated lipid is a PEGylated lipid with the structure:



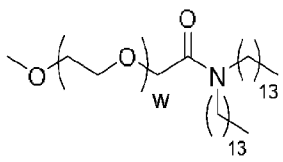
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60.

In some embodiments, the polymer conjugated lipid is a PEGylated lipid with the structure:



,

or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60.

In some embodiments of either structure above, w is an integer ranging from 45 to 55.

In some embodiments, w is about 49.

In some embodiments, the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

In some embodiments, the LNP further comprises a lipid stabilizer.

In some embodiments, the LNP has a molar ratio of the lipid stabilizer to the phospholipid of from 10:1 to 1:4. In some embodiments, the molar ratio of the lipid stabilizer to the phospholipid is from 5:1 to 1:3. In some embodiments, the LNP has a molar ratio of the lipid stabilizer to the phospholipid of from 10:1 to 1:2. In some embodiments, the molar ratio of the lipid stabilizer to the phospholipid is from 5:1 to 1:1. In some embodiments, the molar ratio of the lipid stabilizer to the phospholipid is from 4:1 to 3:1.

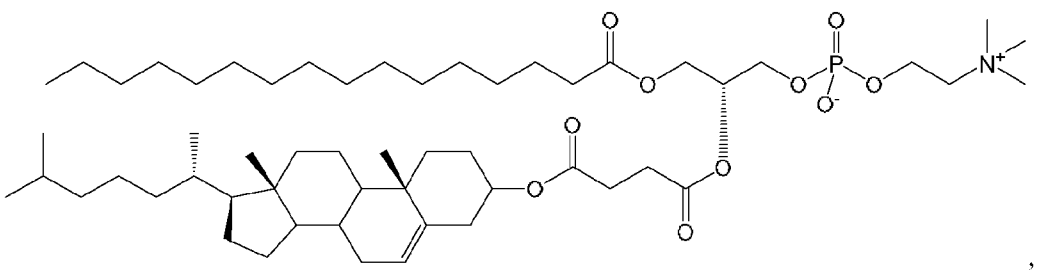
In some embodiments, the lipid stabilizer comprises from 5 to 50 mol% of the total amount of lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 8 to 40 mol% of the total amount of lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 10 to 30 mol% of the total amount of lipids in the LNP.

In some embodiments, the phospholipid comprises from 1 to 30 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 2 to 25 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 3 to 20 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 5 to 15 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises about 10 mol% of the total amount of lipids in the LNP.

In some embodiments, the LNP has a size of from 20 nm to 300 nm, as determined using dynamic light scattering. In some embodiments, the LNP has a size of from 50 nm to 150 nm, as determined using dynamic light scattering. In some embodiments, the size is from 60 nm to 140 nm. In some embodiments, the size is from 80 nm to 100 nm. In some embodiments, the size is from 85 nm to 95 nm.

In some embodiments, the LNP encapsulates mRNA.

Also provided herein are LNPs comprising a phospholipid, wherein the phospholipid has a structure:



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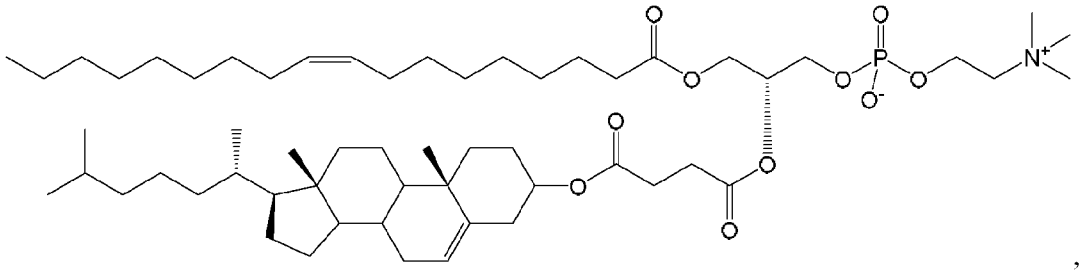

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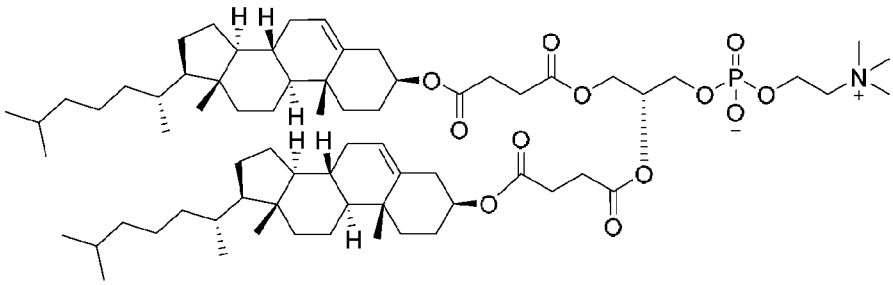
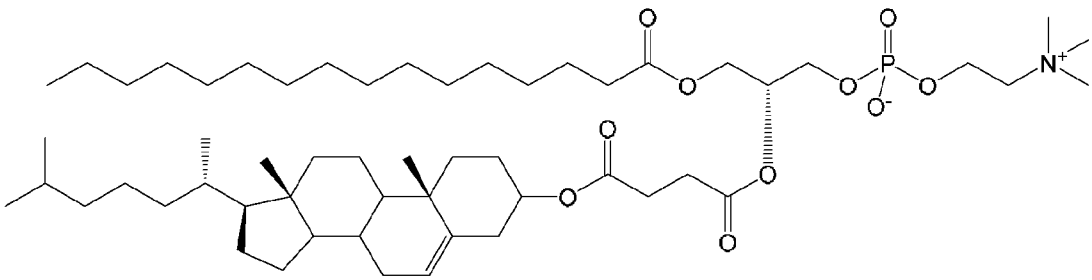
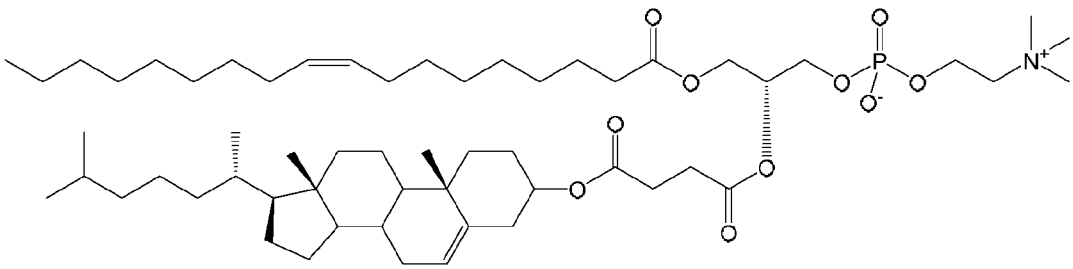


In some embodiments, the phospholipid has the structure:



In some embodiments, the phospholipid has the structure:



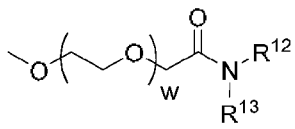
In some embodiments, the LNP further comprises an ionizable lipid.

In some embodiments, the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1. In some embodiments, the molar ratio of ionizable lipid to phospholipid is from 15:1 to 5:1. In some embodiments, the ionizable lipid comprises from 40 to 80 mol% of a total

amount of lipids in the LNP. In some embodiments, the ionizable lipid comprises from 50 to 70 mol% of the total amount of lipids in the LNP. In some embodiments, the ionizable lipid is a compound according to any one of the formula selected from 01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, or wherein the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5. In some embodiments, the ionizable lipid is a cationic lipid.

In some embodiments, the LNP further comprises a polymer conjugated lipid. In some embodiments, the polymer conjugated lipid comprises from 1 to 2 mol% of a total amount of lipids in the LNP. In some embodiments, the polymer conjugated lipid comprises 1.5% of the total amount of lipids in the LNP. In some embodiments, the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid from 1:5 to 1:10. In some embodiments, the polymer conjugated lipid is a PEGylated lipid.

In some embodiments, the polymer conjugated lipid is a PEGylated lipid with the structure:



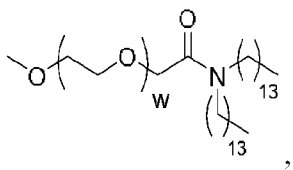
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60.

In some embodiments, the polymer conjugated lipid is a PEGylated lipid with the structure:



,

or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60.

In some embodiments of either structure above, w is an integer ranging from 45 to 55. In some embodiments, w is about 49.

In some embodiments, the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

In some embodiments, the LNP further comprises a lipid stabilizer. In some embodiments, the LNP has a molar ratio of the lipid stabilizer to the phospholipid from 10:1 to 1:4. In some embodiments, the molar ratio of the lipid stabilizer to the phospholipid is from 5:1 to 1:3. In some embodiments, the molar ratio of the lipid stabilizer to the phospholipid is from 4:1 to 3:1. In some embodiments, the lipid stabilizer comprises from 5 to 50 mol% of a total amount of lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 8 to 40 mol% of the total amount of lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 10 to 30 mol% of the total amount of lipids in the LNP.

In some embodiments, the phospholipid comprises from 1 to 30 mol% of a total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 2 to 25 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 3 to 20 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises from 5 to 15 mol% of the total amount of lipids in the LNP. In some embodiments, the phospholipid comprises about 10 mol% of the total amount of lipids in the LNP. In some embodiments, the LNP has a size of from 20 nm to 300 nm, as determined using dynamic light scattering. In some embodiments, the LNP has a size of from 50 nm to 150 nm, as determined using dynamic light scattering. In some embodiments, the size is from 60 nm to 140 nm. In some embodiments, the size is from 80 nm to 100 nm. In some embodiments, the size is from 85 nm to 95 nm.

In some embodiments, the LNP encapsulates mRNA.

Also provided herein are compositions of the lipid nanoparticles (LNPs) described herein. In some embodiments, at least 80% of the LNPs encapsulate mRNA. In some embodiments, at least 85% of the LNPs encapsulate mRNA.

Also provided herein are methods for expressing protein in a cell, comprising introducing an LNP, as described herein, to the cell. In some embodiments, the cell is a mammalian cell.

Also provided herein are methods for delivering a protein to a subject, comprising administering an LNP or composition thereof, as described herein, to the individual, wherein the mRNA encodes the protein. In some embodiments, the LNP or composition thereof is administered systemically. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner.

FIG. 1 shows expression levels of hEPO in LNP formulations containing PChemsPC or OChemsPC with a 60:10:28.5:1.5 molar ratio of compound 01-1/OChemsPC or PChemsPC/Chol/DMG-PEG in Example 15.

FIG. 2 shows expression levels of hEPO in LNP formulations containing DSPC or PChemsPC at various molar percentages in Example 16.

FIG. 3 shows expression levels of hEPO in LNP formulations containing PChemsPC at various molar ratios of PChemsPC/Chol in Example 17.

FIG.4 shows the luminescence levels measured from harvest liver tissues in Example 19.

Fig.5 shows the percentage of luminescence intensity in different tissues in Example 19.

FIG.6 shows hEPO expression fold change of LNPs with or without a steroid containing phospholipid in Example 20.

FIG.7 shows serum cytokines levels boosted by compound 01-1 LNP in Example 21.

FIG.8 shows serum cytokines levels boosted by Lipid 5 LNP in Example 21.

FIG.9 shows serum cytokines levels boosted by SM-102 LNP in Example 21.

FIG.10 shows serum cytokines levels boosted by ALC-0315 LNP in Example 21.

FIG.11 shows serum cytokines levels boosted by Compound 03-135 LNP in Example 21.

FIG.12 shows serum cytokines levels boosted by Compound 01-1 saRNA-LNP in Example 22.

FIG. 13 shows serum cytokines levels boosted by Compound 03-135 saRNA-LNP in Example 22.

FIG. 14 shows CD3-CD19 antibody levels of LNPs with or without a steroid containing phospholipid in Example 23.

DETAILED DESCRIPTION

Provided herein are LNPs comprising a phospholipid containing a sterol moiety. The LNPs can be loaded with mRNA, such as in mRNA vaccine technology. Sterol-modified phospholipids stabilize bilayers but do not exchange between membranes as freely as cholesterol. The mRNA-loaded LNPs demonstrate an ability to increase protein expression in target cells compared to

mRNA-loaded LNPs with more conventional phospholipids. Increasing protein expression helps increase the effectiveness and efficiency of mRNA-based treatments and therapies.

I. Definitions

As used herein, by "pharmaceutically acceptable" or "pharmacologically compatible" is meant a material that is not biologically or otherwise undesirable, *e.g.*, the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

As used herein, "a pharmaceutically acceptable carrier" refers to a pharmaceutically acceptable substrate, composition or vehicle used in the process of drug delivery, which may have one or more ingredients including, but not limited to, excipient(s), binder(s), diluent(s), solvent(s), filler(s), and/or stabilizer(s).

As used herein, the term "lipid" refers to a group of compounds including, without limitation, fats, sterols, waxes, fat-soluble vitamins, monoglycerides, diglycerides, sphingolipids, and phospholipids. In the context of the present disclosure, phospholipids, ionizable lipids, polymer conjugated lipids, and lipid stabilizers are considered lipids.

As used herein, the term "ionizable lipid" refers to a lipid that has a non-zero net electric charge at physiological pH. The term is inclusive with respect to cationic lipids, including lipids that have a partial positive charge at physiological pH. The term is also inclusive with respect to mixtures of ionizable lipids, which can contain two or more ionizable lipids. In every case where an embodiment is contemplated with the term "ionizable lipid," it is likewise contemplated with a "cationic lipid," as though all embodiments were specifically and individually listed with both ionizable and cationic lipids.

As used herein, the term "polymer conjugated lipid" refers to a lipid comprising a polymer moiety. The term is inclusive with respect to PEGylated lipids, including PEGylated phosphatidylethanolamines, PEGylated phosphatidic acids, PEGylated ceramides, PEGylated dialkylamines, PEGylated diacylglycerols, and PEGylated dialkylglycerols. The term is also inclusive with respect to mixture of polymer conjugated lipids, which may contain two or more

polymer conjugated lipids. In every case where an embodiment is contemplated with the term "polymer conjugated lipid," it is likewise contemplated with a "PEGylated lipid," as though all embodiments were specifically and individually listed with both polymer conjugated and PEGylated lipids.

As used herein, the term "lipid stabilizer" refers to a component of the lipid nanoparticle that thought to help stabilize the LNP structure. Without being bound by theory, it is believed that the lipid stabilizer component of LNPs helps favor the liquid-ordered phase of the lipid membrane in LNPs. See, for example, section 3.3.1 of Albertsen, H. C.; et al., "The role of lipid components in lipid nanoparticles for vaccines and gene therapy." Adv Drug Deliv Rev. 2022 Sep; 188:114416. Compounds that can serve as lipid stabilizers include sterols, corticosteroids, vitamins, and other compounds comprising a steroid core.

As used herein, the term "alkyl" refers to a chain of carbon atoms wherein all bonds between the carbon atoms in the alkyl group are single bonds. The term is inclusive with respect to straight and branched chains (*e.g.*, the term includes both n-propyl and isopropyl groups).

As used herein, the term "C_x-C_y alkyl" refers to an alkyl with at least x carbon atoms and no more than y carbon atoms in the alkyl chain. For example, the term "C_1-C_3 alkyl" includes, without limitation, methyl, ethyl, n-propyl, and isopropyl.

As used herein, the term "alkylene" refers to an alkyl chain that connects in at least two locations to other chemical groups. "C_x-C_y alkylene" refers to an alkylene with at least x carbon atoms and no more than y carbon atoms in the alkylene chain. For example, the term "C_1-C_3 alkylene" includes, without limitation, methylene, ethylene, n-propylene, and iso-propylene.

As used herein, the term "alkenyl" refers to a chain of carbon atoms with at least one double bond between two carbon atoms in the chain. The term is inclusive with respect to straight and branched chains (*e.g.*, the term includes both 1-propenyl and iso-propenyl groups).

As used herein, the term "C_x-C_y alkenyl" refers to an alkenyl with at least x carbon atoms and no more than y carbon atoms in the alkenyl chain. For example, the term "C_2-C_4 alkenyl" includes, without limitation, vinyl and 1-propenyl.

As used herein, the term "alkenylene" refers to an alkenyl chain that connects in at least two locations to other chemical groups. "C_x-C_y alkenylene" refers to an alkenylene with at least x carbon atoms and no more than y carbon atoms.

As used herein, the term "alkynyl" refers to a chain of carbon atoms with at least one triple bond between two carbon atoms in the chain. The term is inclusive with respect to straight and branched chains (*e.g.*, the term includes both 1-propynyl and iso-propynyl groups).

As used herein, the term "C_x-C_y alkynyl" refers to an alkynyl with at least x carbon atoms and no more than y carbon atoms in the alkynyl chain.

As used herein, the term "cycloalkyl" refers to a cyclic group of carbon atoms wherein all the bonds between the carbon atoms are single bonds. The term "C_x-C_y cycloalkyl" refers to a cycloalkyl with at least x carbon atoms and no more than y carbon atoms. For example, the term "C_6-C_{10} cycloalkyl" includes, without limitation, cyclohexyl and cyclo-octyl. The term "cycloalkylene" has the same meaning as cycloalkyl, except that the cycloalkylene connects to at least two other chemical groups.

As used herein, the term "cycloalkenyl" refers to a cyclic group of carbon atoms where at least one bond between two carbon atoms in the cycloalkenyl group is a double bond. The term "C_x-C_y cycloalkenyl" refers to a cycloalkenyl with at least x carbon atoms and no more than y carbon atoms.

The term "C_x-C_y aryl" refers to an aryl with at least x carbon atoms and no more than y carbon atoms. For example, the term "C_6-C_{10} aryl" includes, without limitation, phenyl and naphthyl. The term "arylene" has the same meaning as aryl, except that the arylene connects to at least two other chemical groups.

As used herein, the term "heterocycloalkyl" refers to a cyclic group of atoms wherein all the bonds between the atoms in the ring are single bonds. The term "C_x-C_y heterocycloalkyl" refers to a heterocycloalkyl with at least x atoms and no more than y atoms. For example, the term "C_5-C_6 heterocycloalkyl" includes, without limitation, pyrrolidinyl and 1,4-dioxanyl. The term "heterocycloalkylene" has the same meaning as heterocycloalkyl, except that the heterocycloalkylene connects to at least two other chemical groups.

The term "x- to y-membered heteroaryl" refers to a cyclic group of atoms with at least x atoms and no more than y atoms. For example, 5- or 6-membered heteroaryl includes, without limitation, pyridinyl and furanyl.

As used herein, the term "carbocycle" refers to a cycloalkyl or an aryl group. Likewise, the term "heterocycle" refers to a heterocycloalkyl or a heteroaryl group.

Possible atoms that make up the ring in heterocycloalkyl and heteroaryl groups, as well as derivatives thereof, include, without limitation, carbon, nitrogen, oxygen, and sulfur.

As used herein, the term "optionally substituted" means the indicated group may be substituted or unsubstituted. The term substituted refers to another chemical moiety that decorates the indicated group by replacement of one H atom. For example, ethanol is an example of ethane substituted with OH. In some embodiments, a group that is optionally substituted is optionally substituted by chloro, fluoro, bromo, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, C_6-C_{10} aryl, or 5- or 6-membered heteroaryl.

The terms "individual," "subject," and "patient" are used interchangeably herein to describe a mammal, including humans. In some embodiments, the individual is in need of treatment, for example, the individual may have been diagnosed with, or is suspected of having, a cancer.

It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X". In some embodiments, the term "about" a value or parameter means a range within 20%, in either direction, of the value or parameter recited.

As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

As used herein and in the appended claims, the mole percentages of lipids and lipid stabilizers in lipid nanoparticles are calculated based on the total mole number of components in the lipid nanoparticles.

II. Lipid Nanoparticles (LNPs)

The lipid nanoparticles (LNPs) herein comprise a phospholipid containing a sterol moiety and optionally one or more of an ionizable lipid, a polymer conjugated lipid, and a lipid stabilizer. In some embodiments, the LNPs comprise a phospholipid containing a sterol moiety, an ionizable lipid, and a polymer conjugated lipid. In some embodiments, the LNPs comprise a

phospholipid containing a sterol moiety, a polymer conjugated lipid, and a lipid stabilizer. In some embodiments, the LNPs comprise a phospholipid containing a sterol moiety, an ionizable lipid, and a lipid stabilizer. In some embodiments, the LNPs comprise a phospholipid containing a sterol moiety, an ionizable lipid, a polymer conjugated lipid, and a lipid stabilizer.

In some embodiments, the phospholipid comprises from 1 to 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 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, or 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 2 to 25 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 3 to 20 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 5 to 10, 15, 20, 25, or 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 10 to 15, 20, 25, or 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 15 to 20, 25, or 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 20 to 25 or 30 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 25 to 30 mol% of the total lipids in the LNP.

In some embodiments, the ionizable lipid comprises from 40 to 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipid comprises from 40 to 50, 60, 70, or 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipid comprises from 45 to 50, 60, 70, 75, or 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipid comprises from 50 to 60, 70, or 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipid comprises from 60 to 65, 70 or 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipid comprises from 70 to 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipids comprise about 40, 50, 60, 70, or 80 mol% of the total lipids in the LNP. In some embodiments, the ionizable lipids comprise about 50, 60, or 70 mol% of the total lipids in the LNP.

In some embodiments, the LNP has a molar ratio of the ionizable lipid to the phospholipid of from 20:1 to 2:1. In some embodiments, the molar ratio is from 20:1 to 15:1, 10:1, 5:1, or 2:1. In some embodiments, the molar ratio is from 18:1 to 2.5:1. In some embodiments, the molar ratio is from 16:1 to 4:1. In some embodiments, the molar ratio is from 15:1 to 10:1, 5:1, or 2:1.

In some embodiments, the molar ratio is from 10:1 to 5:1 or 2:1. In some embodiments, the ratio is from 5:1 to 2:1. In some embodiments, the ratio is from 15:1 to 5:1.

In some embodiments, the polymer conjugated lipid has a molar ratio of 0.5 to 5 mol% of the total lipids in the LNP. In some embodiments, the polymer conjugated lipid has a molar ratio of 1 to 2 mol% of the total lipids in the LNP. In some embodiments, the polymer conjugated lipid has a molar ratio of 1.5 mol% of the total lipids in the LNP.

In some embodiments, the molar ratio of the polymer conjugated lipid to the phospholipid from 1:2 to 1:20. In some embodiments, the molar ratio of the polymer conjugated lipid to the phospholipid from 1:3 to 1:18. In some embodiments, the molar ratio of the polymer conjugated lipid to the phospholipid from 1:5 to 1:10.

In some embodiments, the lipid stabilizer comprises from 5 to 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 5 to 10, 20, 30, 40, or 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 8 to 20, 30, 40, or 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 10 to 20, 30, 40, or 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 20 to 30, 40, or 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 30 to 40 or 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises from 40 to 50 mol% of the total lipids in the LNP. In some embodiments, the lipid stabilizer comprises about 5, 10, 20, 30, 40, or 50 mol% of the total lipids in the LNP.

It is to be understood that any of the molar percentages or molar ratios described above for the sterol-containing phospholipid, the ionizable lipid, the polymer conjugated lipid, and the lipid stabilizer may be combined with each other in any embodiment describing the lipid composition of the LNPs described herein, such that every combination is contemplated as though each and every combination were specifically and individually disclosed.

In some embodiments, the phospholipid comprises from 1 to 30 mol%, the ionizable lipid comprises from 40 to 80 mol%, the polymer conjugated lipid comprises from 1 to 2 mol%, and the lipid stabilizer comprises from 5 to 50 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises from 5 to 25 mol%, the ionizable lipid comprises from 45 to 75 mol%, the polymer conjugated lipid comprises from 1 to 2 mol%, and the lipid stabilizer comprises from 20 to 40% of the total lipids in the LNP. In some embodiments, the

phospholipid comprises from 5 to 15 mol%, the ionizable lipid comprises from 40 to 60 mol%, the polymer conjugated lipid comprises from 1 to 2 mol%, and the lipid stabilizer comprises from 20 to 40 mol% of the total lipids in the LNP. In some embodiments, the phospholipid comprises about 10 mol%, the ionizable lipid comprises about 50 mol%, the polymer conjugated lipid comprises about 38.5 mol%, and the lipid stabilizer comprises about 1.5 mol% of the total lipids in the LNP.

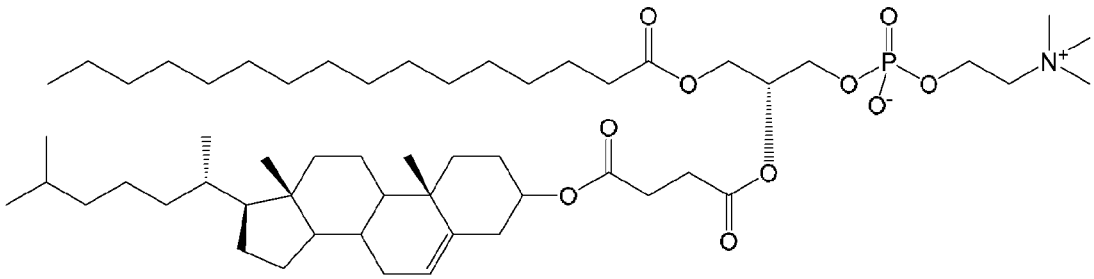
In some embodiments, the LNP has a size from 20 to 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 40 to 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 50 to 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 60 to 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 70 to 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 80 to 90, 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 90 to 100, 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 100 to 110, 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 110 to 120, 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 120 to 130, 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 130 to 140, 150, 200, 250, or 300 nm. In some embodiments, the size is from 140 to 150, 200, 250, or 300 nm. In some embodiments, the size is from 150 to 200, 250, or 300 nm. In some embodiments, the size is from 200 to 300 nm. In some embodiments, the size is from 60 to 150 nm. In some embodiments, the size is from 65 to 90 nm. In some embodiments, the size is from 70 to 80 nm. In some embodiments, the size is about 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, or 115 nm. In some embodiments, the size is about 85 or about 90 nm. In some embodiments, the size is about 85 nm. In some embodiments, the size is about 90 nm.

A. Phospholipid containing a sterol moiety

The LNPs herein comprise a phospholipid containing a sterol moiety. The phospholipid containing a sterol moiety is any phospholipid that incorporates a sterol moiety into the lipid structure. In some embodiments, the sterol moiety is incorporated in place of one or more alkyl chains on the phospholipid. In some embodiments, the sterol moiety is incorporated into one alkyl chain of the phospholipid. In some embodiments, the sterol moiety is connected through the O atom of the sterol (*e.g.* converting the sterol moiety into the -O- atom of an ester

connection to the remainder of the phospholipid). In some embodiments, the sterol moiety is cholesterol. In some embodiments, the sterol moiety is a cholesterol moiety connected through O atom of the sterol (*e.g.* by converting the sterol O atom of cholesterol into the -O- atom of an ester connection to the remainder of the phospholipid).

In some embodiments, the phospholipid has a structure of selected from:



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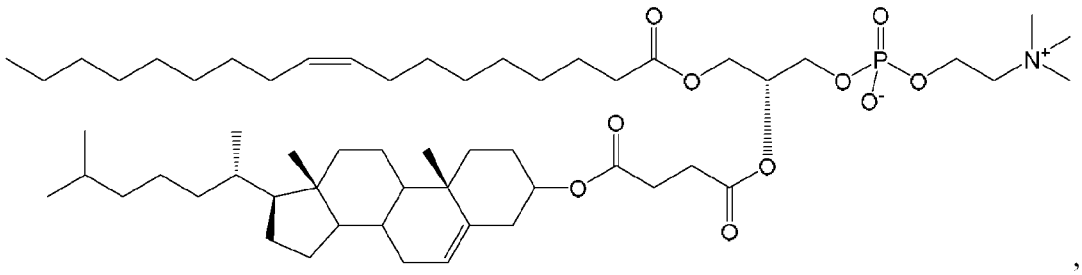
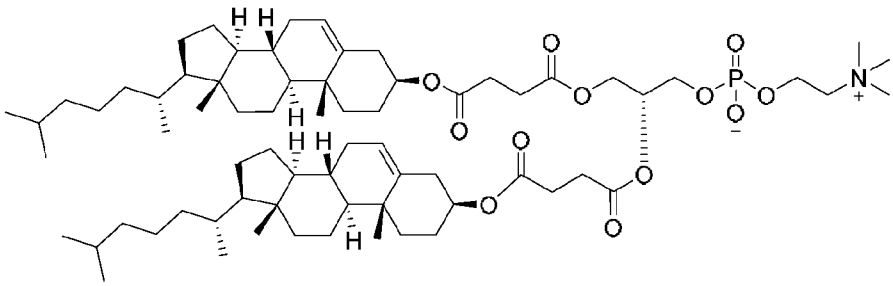


,

and



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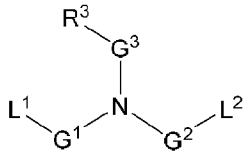
B. Ionizable lipids

In some embodiments, the LNPs comprise an ionizable lipid. In some embodiments, the ionizable lipid is a cationic lipid. In some embodiments, the cationic lipid is a cationic lipid described in International Patent Publication No. WO 2021/204175, the entirety of which is incorporated herein by reference.

In some embodiments, the cationic lipid is a compound of Formula (01-I):



(01-I),

or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein:

G^1 and G^2 are each independently a bond, C_2-C_{12} alkylene, or C_2-C_{12} alkenylene, wherein one or more -CH_2- in the alkylene or alkenylene is optionally replaced by -O-;

L^1 is –OC(=O)R^1, -C(=O)OR^1, -OC(=O)OR^1, -C(=O)R^1, -OR^1, -S(O)xR^1, -S-SR^1, -C(=O)SR^1, -SC(=O)R^1, -NR^aC(=O)R^1, -C(=O)N$R^b$$R^c$, -N$R^a$C(=O)N$R^b$$R^c$, -OC(=O)N$R^b$$R^c$, -N$R^a$C(=O)O$R^1$, -SC(=S)$R^1$, -C(=S)S$R^1$, -C(=S)$R^1$, -CH(OH)$R^1$, -P(=O)(O$R^b$)(O$R^c$), -($C_6$-$C_{10}$ arylene)-R^1, -(6- to 10-membered heteroarylene)-R^1, or R^1;

L^2 is –OC(=O)R^2, -C(=O)OR^2, -OC(=O)OR^2, -C(=O)R^2, -OR^2, -S(O)$_x$$R^2$, -S-S$R^2$, -C(=O)S$R^2$, -SC(=O)$R^2$, -N$R^d$C(=O)$R^2$, -C(=O)N$R^e$$R^f$, -N$R^d$C(=O)N$R^e$$R^f$, -OC(=O)N$R^e$$R^f$, -N$R^d$C(=O)O$R^2$, -SC(=S)$R^2$, -C(=S)S$R^2$, -C(=S)$R^2$, -CH(OH)$R^2$, -P(=O)(O$R^e$)(O$R^f$), -($C_6$-$C_{10}$ arylene)-R^2, -(6- to 10-membered heteroarylene)-R^2, or R^2;

R^1 and R^2 are each independently C_6-C_{32} alkyl or C_6-C_{32} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{32} alkyl or C_2-C_{32} alkenyl;

G^3 is C_2-C_{24} alkylene, C_2-C_{24} alkenylene, C_3-C_8 cycloalkylene, or C_3-C_8 cycloalkenylene;

R^3 is -N(R^4)R^5;

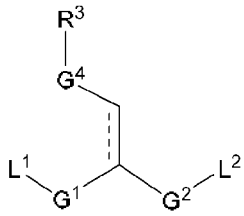
R^4 is C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, 4- to 8-membered heterocyclyl, or C_6-C_{10} aryl; or R^4, G^3 or part of G^3, together with the nitrogen to which they are attached form a cyclic moiety;

R^5 is C_1-C_{12} alkyl or C_3-C_8 cycloalkyl; or R^4, R^5, together with the nitrogen to which they are attached form a cyclic moiety;

x is 0, 1, or 2; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

In some embodiments, the cationic lipid is a compound of Formula (01-II):



(01-II),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

------ is a single bond or a double bond;

G^1 and G^2 are each independently a bond, C_2-C_{12} alkylene, or C_2-C_{12} alkenylene, wherein one or more -CH_2- in the alkylene or alkenylene is optionally replaced by -O-;

L^1 is –OC(=O)R^1, -C(=O)OR^1, -OC(=O)OR^1, -C(=O)R^1, -OR^1, -S(O)$_x$$R^1$, -S-S$R^1$, -C(=O)S$R^1$, -SC(=O)$R^1$, -N$R^a$C(=O)$R^1$, -C(=O)N$R^b$$R^c$, -N$R^a$C(=O)N$R^b$$R^c$, -OC(=O)N$R^b$$R^c$, -N$R^a$C(=O)O$R^1$, -SC(=S)$R^1$, -C(=S)S$R^1$, -C(=S)$R^1$, -CH(OH)$R^1$, -P(=O)(O$R^b$)(O$R^c$), -($C_6$-$C_{10}$ arylene)-R^1, -(6- to 10-membered heteroarylene)-R^1, or R^1;

L^2 is –OC(=O)R^2, -C(=O)OR^2, -OC(=O)OR^2, -C(=O)R^2, -OR^2, -S(O)$_x$$R^2$, -S-S$R^2$, -C(=O)S$R^2$, -SC(=O)$R^2$, -N$R^d$C(=O)$R^2$, -C(=O)N$R^e$$R^f$, -N$R^d$C(=O)N$R^e$$R^f$, -OC(=O)N$R^e$$R^f$, -N$R^d$C(=O)O$R^2$, -SC(=S)$R^2$, -C(=S)S$R^2$, -C(=S)$R^2$, -CH(OH)$R^2$, -P(=O)(O$R^e$)(O$R^f$), -($C_6$-$C_{10}$ arylene)-R^2, -(6- to 10-membered heteroarylene)-R^2, or R^2;

R^1 and R^2 are each independently C_6-C_{32} alkyl or C_6-C_{32} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{32} alkyl or C_2-C_{32} alkenyl;

G^4 is a bond, C_1-C_{23} alkylene, C_2-C_{23} alkenylene, C_3-C_8 cycloalkylene, or C_3-C_8 cycloalkenylene;

R^3 is -N(R^4)R^5;

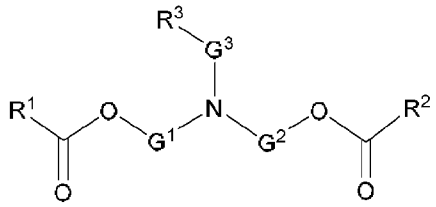
R^4 is C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, 4- to 8-membered heterocyclyl, or C_6-C_{10} aryl; or R^4, G^3 or part of G^3, together with the nitrogen to which they are attached form a cyclic moiety;

R^5 is C_1-C_{12} alkyl or C_3-C_8 cycloalkyl; or R^4 and R^5, together with the nitrogen to which they are attached form a cyclic moiety;

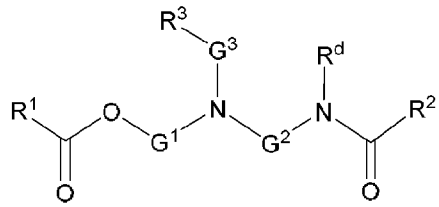
x is 0, 1, or 2; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

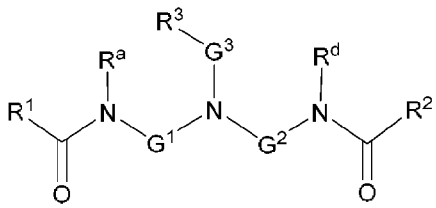
In some embodiments, the cationic lipid is a compound of Formula (01-I-B), (01-I-B'), (01-I-B"), (01-I-C), (01-I-D), or (01-I-E):



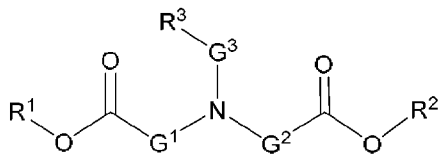
(01-I-B),



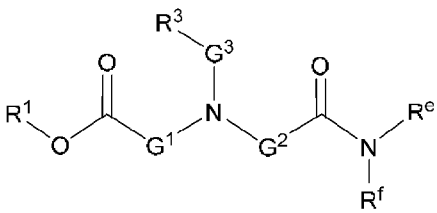
(01-I-B'),



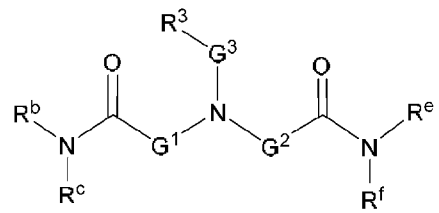
(01-I-B"),



(01-I-C),



(01-I-D), or

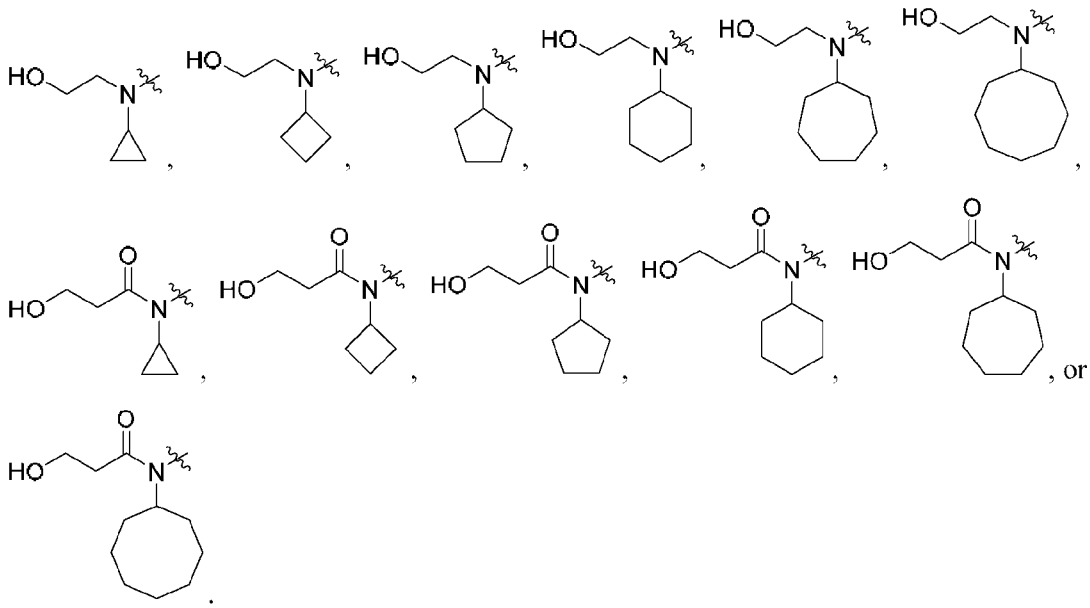


(01-I-E),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

In some embodiments, G^1 and G^2 are each independently C_3-C_7 alkylene. In some embodiments, G^1 and G^2 are each independently C_5 alkylene. In some embodiments, G^3 is C_2-C_4 alkylene. In some embodiments, G^3 is C_2 alkylene. In some embodiments, G^3 is C_4 alkylene.

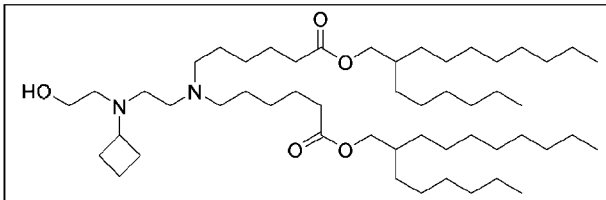
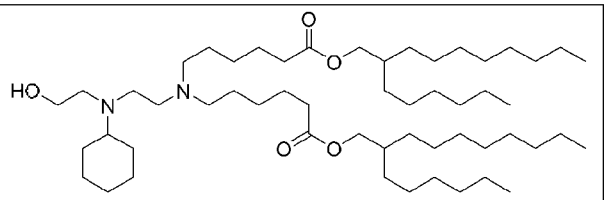
In some embodiments, R^3 has one of the following structures:

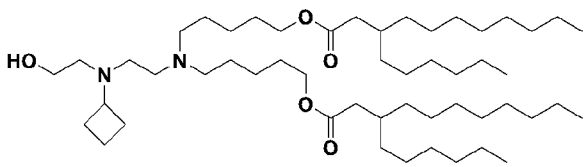
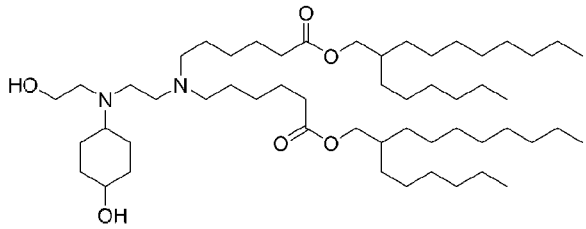
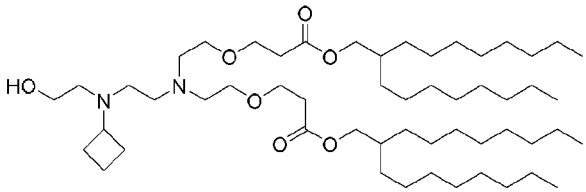
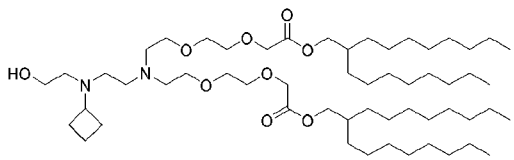
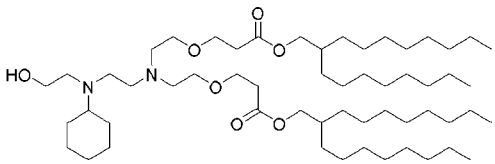
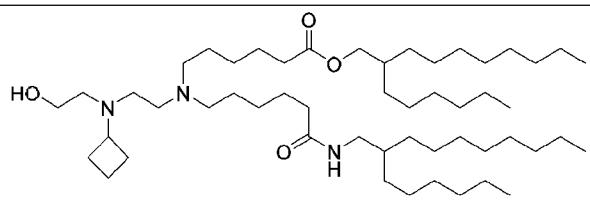
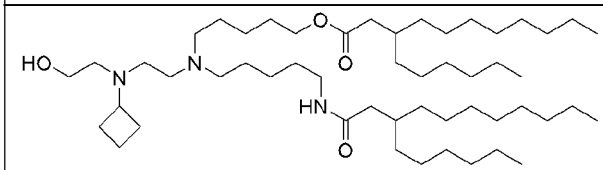
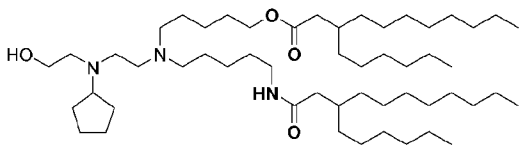
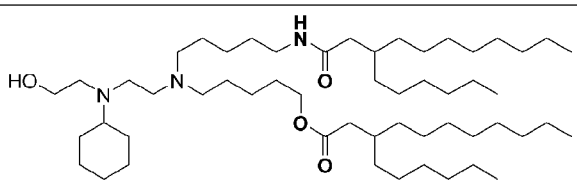
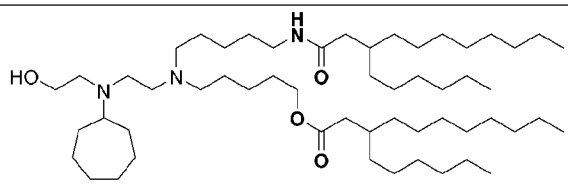
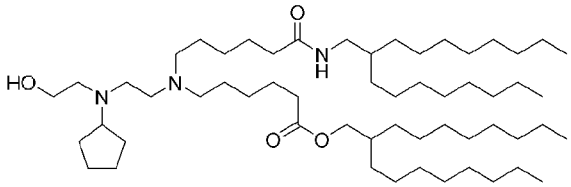


In some embodiments, R^1, R^2, R^e, and R^f are each independently branched C_6-C_{32} alkyl or branched C_6-C_{32} alkenyl. In some embodiments, R^1, R^2, R^e, and R^f are each independently branched C_6-C_{24} alkyl or branched C_6-C_{24} alkenyl. In some embodiments, R^1, R^2, R^e, and R^f are each independently $-R^7-CH(R^8)(R^9)$, wherein R^7 is C_0-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl. In some embodiments, R^1, R^2, R^e, and R^f are each independently $-R^7-CH(R^8)(R^9)$, wherein R^7 is C_0-C_1 alkylene, and R^8 and R^9 are independently C_4-C_8 alkyl.

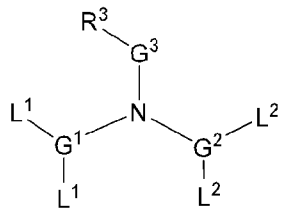
In some embodiments, the cationic lipid is a compound in Table 1, or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

Table 1.

 Compound 01-1	 Compound 01-2

 Compound 01-20	 Compound 01-21
 Compound 01-102	 Compound 01-103
 Compound 01-104	 Compound 01-106
 Compound 01-108	 Compound 01-130
 Compound 01-131	 Compound 01-132
 Compound 01-136	

In some embodiments, the cationic lipid is a cationic lipid described in International Patent Application No. PCT/CN2022/072694, the entirety of which is incorporated herein by reference. In some embodiments, the cationic lipid is a compound of Formula (02-I):



(02-I),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

G^1 and G^2 are each independently C_2-C_{12} alkylene or C_2-C_{12} alkenylene, wherein one or more $-CH_2-$ in G^1 and G^2 is optionally replaced by -O-, -C(=O)O-, or -OC(=O)-;

each L^1 is independently $-OC(=O)R^1$, $-C(=O)OR^1$, $-OC(=O)OR^1$, $-C(=O)R^1$, $-OR^1$, $-S(O)_xR^1$, $-S-SR^1$, $-C(=O)SR^1$, $-SC(=O)R^1$, $-NR^aC(=O)R^1$, $-C(=O)NR^bR^c$, $-NR^aC(=O)NR^bR^c$, $-OC(=O)NR^bR^c$, $-NR^aC(=O)OR^1$, $-SC(=S)R^1$, $-C(=S)SR^1$, $-C(=S)R^1$, $-CH(OH)R^1$, $-P(=O)(OR^b)(OR^c)$, $-NR^aP(=O)(OR^b)(OR^c)$;

each L^2 is independently $-OC(=O)R^2$, $-C(=O)OR^2$, $-OC(=O)OR^2$, $-C(=O)R^2$, $-OR^2$, $-S(O)_xR^2$, $-S-SR^2$, $-C(=O)SR^2$, $-SC(=O)R^2$, $-NR^dC(=O)R^2$, $-C(=O)NR^eR^f$, $-NR^dC(=O)NR^eR^f$, $-OC(=O)NR^eR^f$, $-NR^dC(=O)OR^2$, $-SC(=S)R^2$, $-C(=S)SR^2$, $-C(=S)R^2$, $-CH(OH)R^2$, $-P(=O)(OR^e)(OR^f)$, $-NR^dP(=O)(OR^e)(OR^f)$;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{24} alkyl or C_2-C_{24} alkenyl;

G^3 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by a C_3-C_8 cycloalkylene or C_3-C_8 cycloalkenylene;

R^3 is $-N(R^4)R^5$, $-OR^6$, or $-SR^6$;

R^4 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

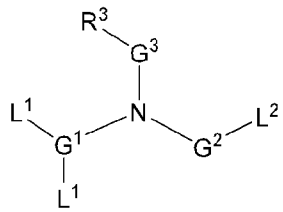
R^5 is H, C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

R^6 is hydrogen, C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, or C_6-C_{10} aryl;

x is 0, 1, or 2; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, cycloalkylene, and cycloalkenylene is independently optionally substituted.

In some embodiments, the cationic lipid is a compound of Formula (02-II):



(02-II),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

G^1 and G^2 are each independently C_2-C_{12} alkylene or C_2-C_{12} alkenylene, wherein one or more $-CH_2-$ in G^1 and G^2 is optionally replaced by -O-, -C(=O)O-, or -OC(=O)-;

each L^1 is independently $-OC(=O)R^1$, $-C(=O)OR^1$, $-OC(=O)OR^1$, $-C(=O)R^1$, $-OR^1$, $-S(O)_xR^1$, $-S-SR^1$, $-C(=O)SR^1$, $-SC(=O)R^1$, $-NR^aC(=O)R^1$, $-C(=O)NR^bR^c$, $-NR^aC(=O)NR^bR^c$, $-OC(=O)NR^bR^c$, $-NR^aC(=O)OR^1$, $-SC(=S)R^1$, $-C(=S)SR^1$, $-C(=S)R^1$, $-CH(OH)R^1$, $-P(=O)(OR^b)(OR^c)$, $-NR^aP(=O)(OR^b)(OR^c)$;

each L^2 is independently $-OC(=O)R^2$, $-C(=O)OR^2$, $-OC(=O)OR^2$, $-C(=O)R^2$, $-OR^2$, $-S(O)_xR^2$, $-S-SR^2$, $-C(=O)SR^2$, $-SC(=O)R^2$, $-NR^dC(=O)R^2$, $-C(=O)NR^eR^f$, $-NR^dC(=O)NR^eR^f$, $-OC(=O)NR^eR^f$, $-NR^dC(=O)OR^2$, $-SC(=S)R^2$, $-C(=S)SR^2$, $-C(=S)R^2$, $-CH(OH)R^2$, $-P(=O)(OR^e)(OR^f)$, $-NR^dP(=O)(OR^e)(OR^f)$;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{24} alkyl or C_2-C_{24} alkenyl;

G^3 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by a C_3-C_8 cycloalkylene or C_3-C_8 cycloalkenylene;

R^3 is $-N(R^4)R^5$, $-OR^6$, or $-SR^6$;

R^4 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

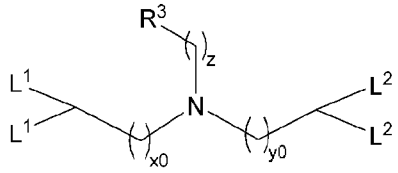
R^5 is H, C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

R^6 is hydrogen, C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, or C_6-C_{10} aryl;

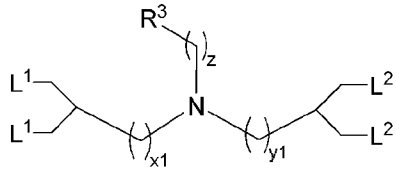
x is 0, 1, or 2; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, cycloalkylene, and cycloalkenylene is independently optionally substituted.

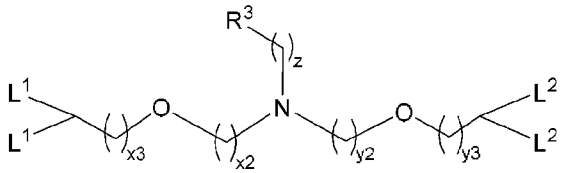
In some embodiments, the compound is a compound of Formula (02-V-A), (02-V-B), (02-V-C), (02-V-D), (02-V-E), (02-V-F):



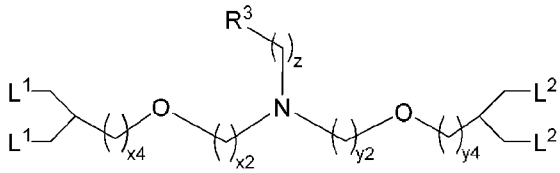
(02-V-A),



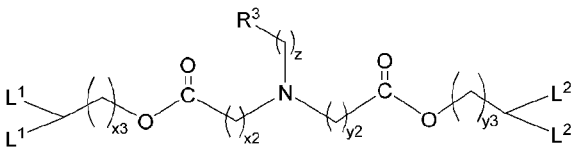
(02-V-B),



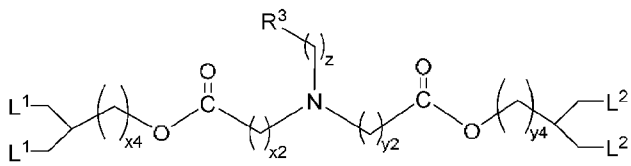
(02-V-C),



(02-V-D),



(02-V-E), or



(02-V-F),

wherein z is an integer from 2 to 12,

x0 is an integer from 1 to 11;

y0 is an integer from 1 to 11;

x1 is an integer from 0 to 9;

y1 is an integer from 0 to 9;

x2 is an integer from 2 to 9;

x3 is an integer from 1 to 5;

x4 is an integer from 0 to 3;

y2 is an integer from 2 to 9;

y3 is an integer from 1 to 5; and

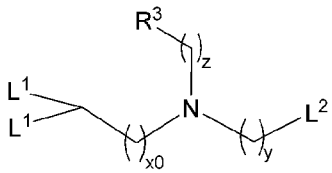
y4 is an integer from 0 to 3;

or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof.

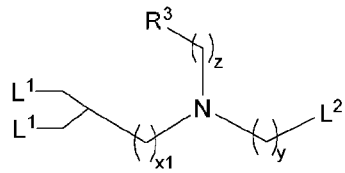
In some embodiments, z is an integer from 2 to 6. In some embodiments, z is 2, 4, or 5. In some embodiments, x0 and y0 are independently 2 to 6. In some embodiments, x0 and y0 are independently 4 or 5. In some embodiments, x1 and y1 are independently 2 to 6. In some embodiments, x1 and y1 are independently 4 or 5. In some embodiments, x2 and y2 are independently an integer from 2 to 8. In some embodiments, x2 and y2 are independently 3, 5, or 7. In some embodiments, x3 and y3 are both 1. In some embodiments, x4 and y4 are independently 0 or 1.

In some embodiments, each L^1 is independently $-OR^1$, $-OC(=O)R^1$, or $-C(=O)OR^1$, and each L^2 is independently $-OR^2$, $-OC(=O)R^2$, or $-C(=O)OR^2$. In some embodiments, R^1 and R^2 are independently straight C_6-C_{10} alkyl, or $-R^7$-$CH(R^8)(R^9)$, wherein R^7 is C_0-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl or C_2-C_{10} alkenyl.

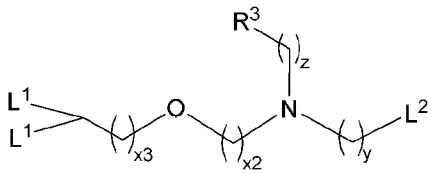
In some embodiments, the compound is a compound of formula (02-VI-A), (02-VI-B), (02-VI-C), (02-VI-D), (02-VI-E), or (02-VI-F):



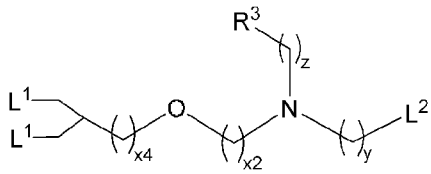
(02-VI-A),



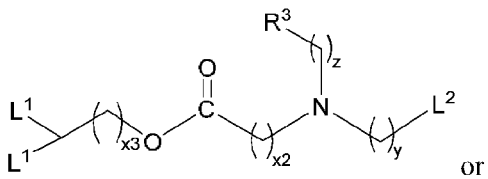
(02-VI-B),



(02-VI-C),

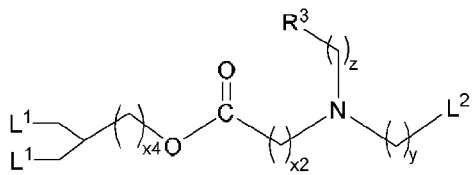


(02-VI-D),



or

(02-VI-E),



(02-VI-F),

wherein z is an integer from 2 to 12;

y is an integer from 2 to 12;

x0 is an integer from 1 to 11;

x1 is an integer from 0 to 9;

x2 is an integer from 2 to 5;

x3 is an integer from 1 to 5; and

x4 is an integer from 0 to 3;

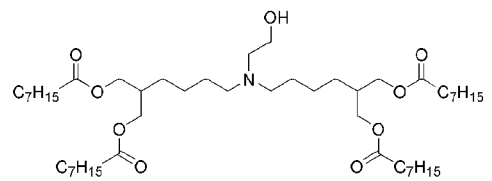
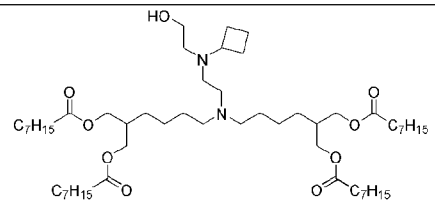
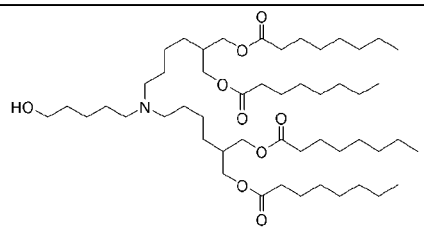
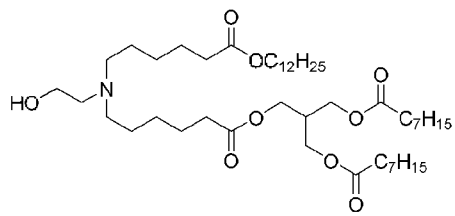
or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

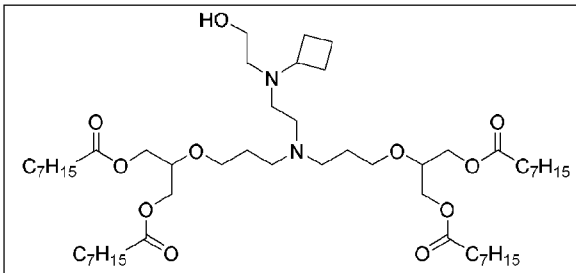
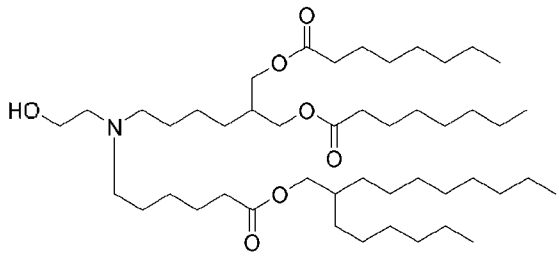
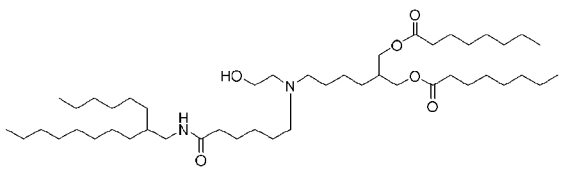
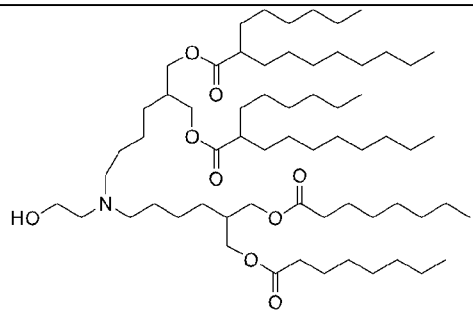
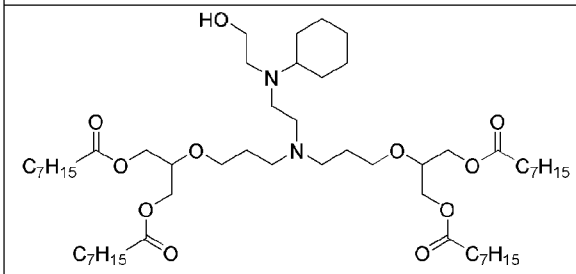
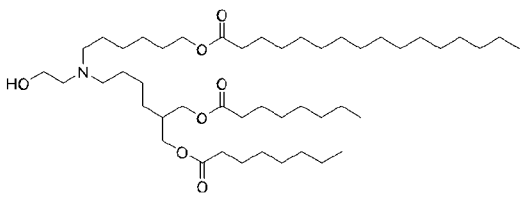
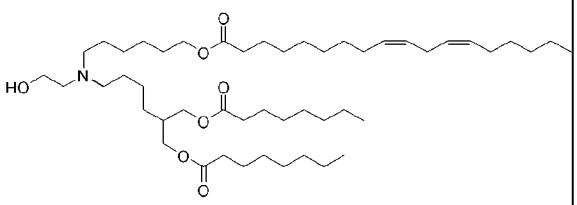
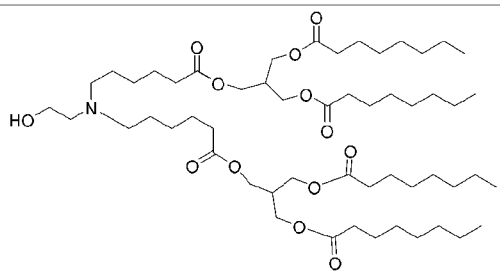
In some embodiments, z is an integer from 2 to 6. In some embodiments, z is 2, 4, or 5. In some embodiments, x0 is 4 or 5. In some embodiments, x1 is 4 or 5. In some embodiments, x2 is an integer from 2 to 5. In some embodiments, x2 is 3 or 5. In some embodiments, x3 is 0 or 1. In some embodiments, y is an integer from 2 to 6. In some embodiments, y is 5.

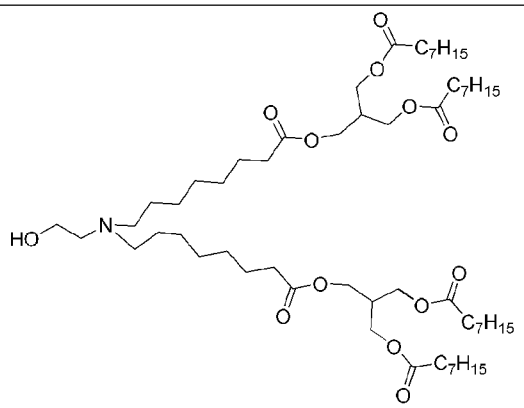
In some embodiments, each L^1 is independently $-OR^1$, $-OC(=O)R^1$ or $-C(=O)OR^1$, and L^2 is $-OC(=O)R^2$ or $-C(=O)OR^2$, $-NR^dC(=O)R^2$, or $-C(=O)NR^eR^f$. In some embodiments, R^1 is straight C_6-C_{10} alkyl or $-R^7$-$CH(R^8)(R^9)$, wherein R^7 is C_0-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl or C_2-C_{10} alkenyl. In some embodiments, R^2 and R^f are each independently straight C_6-C_{18} alkyl, C_6-C_{18} alkenyl, or $-R^7$-$CH(R^8)(R^9)$, wherein R^7 is C_0-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl or C_2-C_{10} alkenyl. In some embodiments, R^d and R^e are each independently H.

In some embodiments, the compound is a compound in Table 2, or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

Table 2.

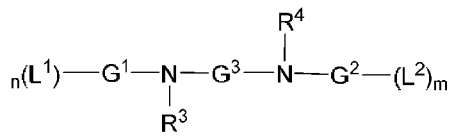
 Compound 02-1	 Compound 02-2
 Compound 02-3	 Compound 02-4

 Compound 02-9	 Compound 02-10
 Compound 02-11	 Compound 02-12
 Compound 02-14	 Compound 02-20
 Compound 02-22	 Compound 02-6

 Compound 02-65	

In some embodiments, the cationic lipid described herein is a cationic lipid described in International Patent Publication No. WO 2022/152109, the entirety of which is incorporated herein by reference.

In some embodiments, the cationic lipid is a compound of Formula (03-I):



(03-I),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

G^1 and G^2 are each independently a bond, C_2-C_{12} alkylene, or C_2-C_{12} alkenylene, wherein one or more $-CH_2-$ in G^1 and G^2 is optionally replaced by -O-;

each L^1 is independently $-OC(=O)R^1$, $-C(=O)OR^1$, $-OC(=O)OR^1$, $-C(=O)R^1$, $-OR^1$, $-S(O)_xR^1$, $-S\text{-}SR^1$, $-C(=O)SR^1$, $-SC(=O)R^1$, $-NR^aC(=O)R^1$, $-C(=O)NR^bR^c$, $-NR^aC(=O)NR^bR^c$, $-OC(=O)NR^bR^c$, $-NR^aC(=O)OR^1$, $-SC(=S)R^1$, $-C(=S)SR^1$, $-C(=S)R^1$, $-CH(OH)R^1$, $-P(=O)(OR^b)(OR^c)$, $-NR^aP(=O)(OR^b)(OR^c)$, -(C_6-C_{10} arylene)-R^1, -(6- to 10-membered heteroarylene)-R^1, -(4- to 8-membered heterocyclylene)-R^1, or R^1;

each L^2 is independently $-OC(=O)R^2$, $-C(=O)OR^2$, $-OC(=O)OR^2$, $-C(=O)R^2$, $-OR^2$, $-S(O)_xR^2$, $-S\text{-}SR^2$, $-C(=O)SR^2$, $-SC(=O)R^2$, $-NR^dC(=O)R^2$, $-C(=O)NR^eR^f$, $-NR^dC(=O)NR^eR^f$, $-OC(=O)NR^eR^f$, $-NR^dC(=O)OR^2$, $-SC(=S)R^2$, $-C(=S)SR^2$, $-C(=S)R^2$, $-CH(OH)R^2$, $-P(=O)(OR^e)(OR^f)$, $-NR^dP(=O)(OR^e)(OR^f)$, -(C_6-C_{10} arylene)-R^2, -(6- to 10-membered heteroarylene)-R^2, -(4- to 8-membered heterocyclylene)-R^2, or R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{24} alkyl or C_2-C_{24} alkenyl;

G^3 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene, wherein part or all of alkylene or alkenylene is optionally replaced by C_3-C_8 cycloalkylene, C_3-C_8 cycloalkenylene, C_3-C_8 cycloalkynylene, 4- to 8-membered heterocyclylene, C_6-C_{10} arylene, or 5- to 10-membered heteroarylene;

R^3 is hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_3-C_8 cycloalkynyl, 4- to 8-membered heterocyclyl, C_6-C_{10} aryl, or 5- to 10-membered heteroaryl; or R^3 and G^1, or part of G^1, together with the nitrogen to which they are attached form a cyclic moiety; or R^3 and G^3 or part of G^3, together with the nitrogen to which they are attached form a cyclic moiety;

R^4 is C_1-C_{12} alkyl or C_3-C_8 cycloalkyl;

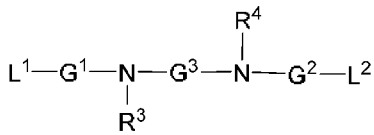
x is 0, 1, or 2;

n is 1 or 2;

m is 1 or 2; and

wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocyclyl, aryl, heteroaryl, alkylene, alkenylene, cycloalkylene, cycloalkenylene, cycloalkynylene, heterocyclylene, arylene, heteroarylene, and cyclic moiety is independently optionally substituted.

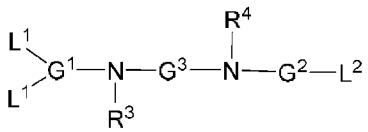
In some embodiments, the cationic lipid is a compound of Formula (03-II-A):



(03-II-A),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

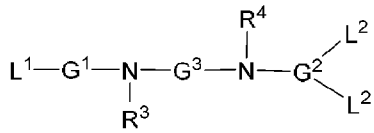
In some embodiments, the cationic lipid is a compound of Formula (03-II-B):



(03-II-B),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

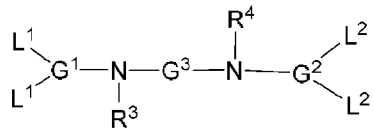
In some embodiments, the cationic lipid is a compound of Formula (03-II-C):



(03-II-C),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

In some embodiments, the cationic lipid is a compound of Formula (03-II-D):



(03-II-D),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

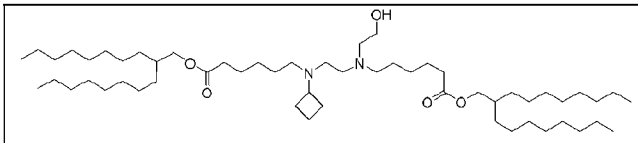
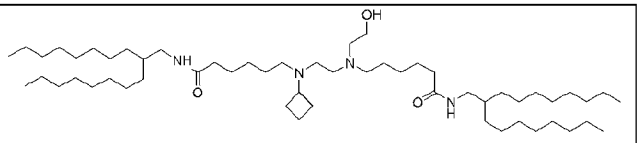
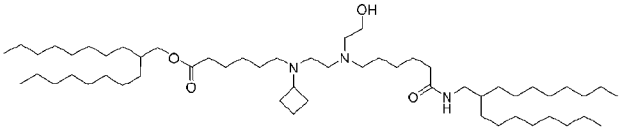
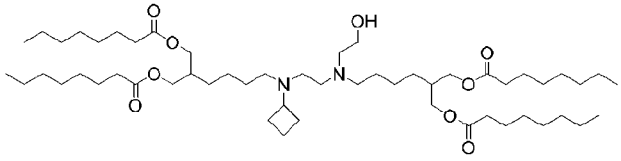
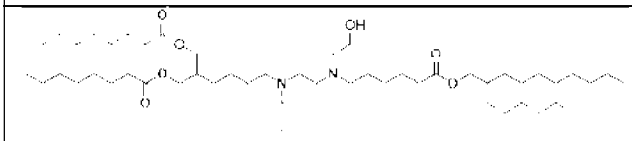
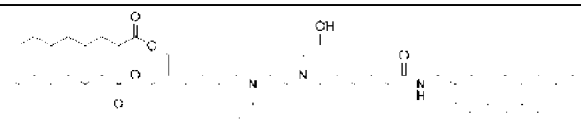
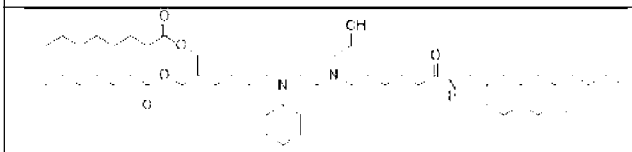
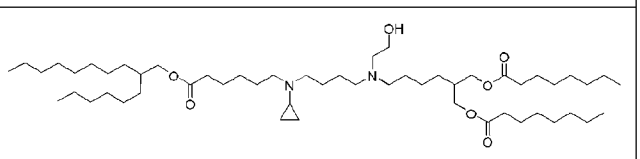
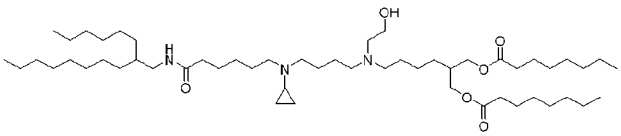
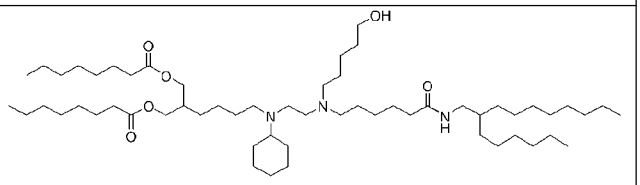
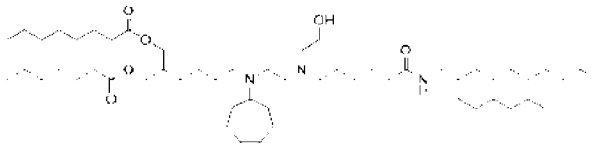
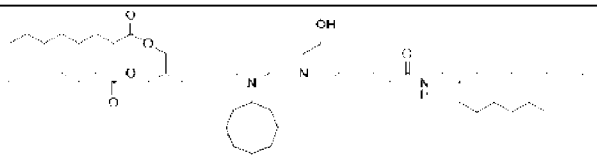
In some embodiments, G^1 and G^2 are each independently C_2-C_{12} alkylene. In some embodiments, G^1 and G^2 are each independently C_5 alkylene. In some embodiments, G^3 is C_2-C_6 alkylene.

In some embodiments, R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, or C_3-C_8 cycloalkyl. In some embodiments, R^3 is C_3-C_8 cycloalkyl. In some embodiments, R^3 is unsubstituted. In some embodiments, R^4 is substituted C_1-C_{12} alkyl. In some embodiments, R^4 is $–CH_2CH_2OH$.

In some embodiments, L^1 is $–OC(=O)R^1$, $-C(=O)OR^1$, $-NR^aC(=O)R^1$, or $-C(=O)NR^bR^c$; and L^2 is $–OC(=O)R^2$, $-C(=O)OR^2$, $-NR^dC(=O)R^2$, or $-C(=O)NR^eR^f$. In some embodiments, R^1, R^2, R^c, and R^f are each independently straight C_6-C_{18} alkyl, straight C_6-C_{18} alkenyl, or $-R^7$-$CH(R^8)(R^9)$, wherein R^7 is C_0-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl or C_2-C_{10} alkenyl. In some embodiments, R^1, R^2, R^c, and R^f are each independently straight C_7-C_{15} alkyl, straight C_7-C_{15} alkenyl, or $-R^7$-$CH(R^8)(R^9)$, wherein R^7 is C_0-C_1 alkylene, and R^8 and R^9 are independently C_4-C_8 alkyl or C_6-C_{10} alkenyl. In some embodiments, R^a, R^b, R^d, and R^e are each independently H.

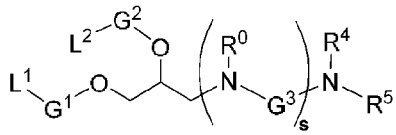
In some embodiments, the cationic lipid is a compound in Table 3, or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

Table 3

 Compound 03-10	 Compound 03-11
 Compound 03-15	 Compound 03-71
 Compound 03-135	 Compound 03-136
 Compound 03-140	 Compound 03-190
 Compound 03-195	 Compound 03-209
 Compound 03-143	 Compound 03-145

In some embodiments, the cationic lipid is a cationic lipid described in International Patent Application No. PCT/CN2022/094227, the entirety of which is incorporated herein by reference.

In some embodiments, the cationic lipid is a compound of Formula (04-I):



(04-I),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

G^1 and G^2 are each independently a bond, C_2-C_{12} alkylene, or C_2-C_{12} alkenylene;

L^1 is $-OC(=O)R^1$, $-C(=O)OR^1$, $-OC(=O)OR^1$, $-C(=O)R^1$, $-OR^1$, $-S(O)_xR^1$, $-S-SR^1$, $-C(=O)SR^1$, $-SC(=O)R^1$, $-NR^aC(=O)R^1$, $-C(=O)NR^bR^c$, $-NR^aC(=O)NR^bR^c$, $-OC(=O)NR^bR^c$, $-NR^aC(=O)OR^1$, $-SC(=S)R^1$, $-C(=S)SR^1$, $-C(=S)R^1$, $-CH(OH)R^1$, $-P(=O)(OR^b)(OR^c)$, -(C_6-C_{10} arylene)-R^1, -(6- to 10-membered heteroarylene)-R^1, or R^1;

L^2 is $-OC(=O)R^2$, $-C(=O)OR^2$, $-OC(=O)OR^2$, $-C(=O)R^2$, $-OR^2$, $-S(O)_xR^2$, $-S-SR^2$, $-C(=O)SR^2$, $-SC(=O)R^2$, $-NR^dC(=O)R^2$, $-C(=O)NR^eR^f$, $-NR^dC(=O)NR^eR^f$, $-OC(=O)NR^eR^f$, $-NR^dC(=O)OR^2$, $-SC(=S)R^2$, $-C(=S)SR^2$, $-C(=S)R^2$, $-CH(OH)R^2$, $-P(=O)(OR^e)(OR^f)$, -(C_6-C_{10} arylene)-R^2, -(6- to 10-membered heteroarylene)-R^2, or R^2;

R^1 and R^2 are each independently C_5-C_{32} alkyl or C_5-C_{32} alkenyl;

R^a, R^b, R^d, and R^e are each independently H, C_1-C_{24} alkyl, or C_2-C_{24} alkenyl;

R^c and R^f are each independently C_1-C_{32} alkyl or C_2-C_{32} alkenyl;

R^0 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

G^3 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene;

R^4 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

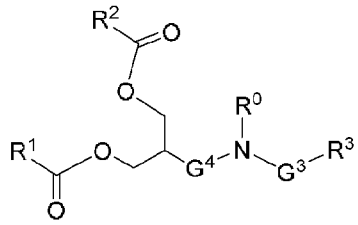
R^5 is C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

x is 0, 1, or 2;

s is 0 or 1; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, arylene, and heteroarylene, is independently optionally substituted.

In some embodiments, the cationic lipid is a compound of Formula (04-III):



(04-III),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof, wherein:

R^1 and R^2 are each independently C_5-C_{32} alkyl or C_5-C_{32} alkenyl;

R^0 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

G^3 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene;

G^4 is C_2-C_{12} alkylene or C_2-C_{12} alkenylene;

R^3 is $-N(R^4)R^5$ or $-OR^6$;

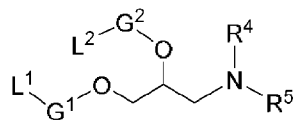
R^4 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl;

R^5 is C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, C_6-C_{10} aryl, or 4- to 8-membered heterocycloalkyl; or R^4 and R^5, together with the nitrogen to which they are attached form a cyclic moiety;

R^6 is hydrogen, C_1-C_{12} alkyl, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkenyl, or C_6-C_{10} aryl; and

wherein each alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, alkylene, alkenylene, and cyclic moiety is independently optionally substituted.

In some embodiments, the cationic lipid is a compound of Formula (04-IV):



(04-IV),

or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

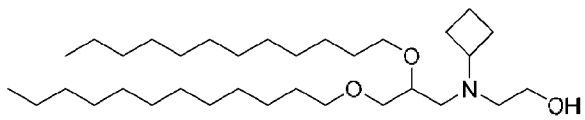
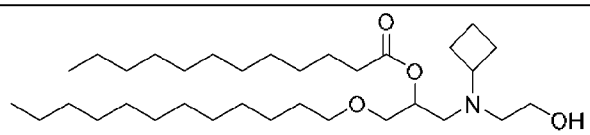
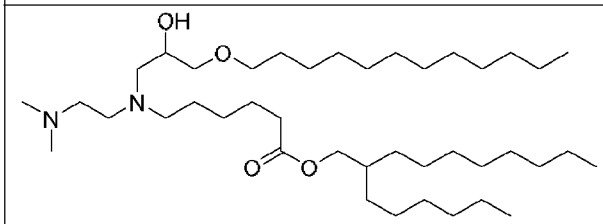
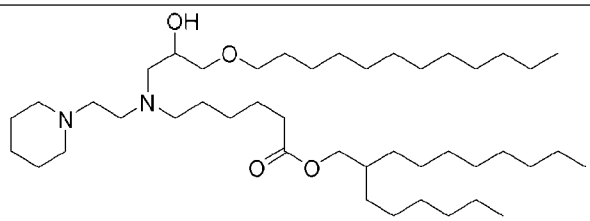
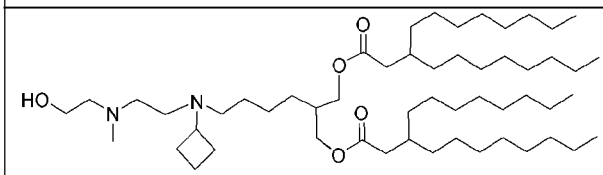
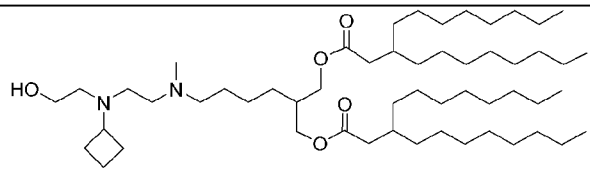
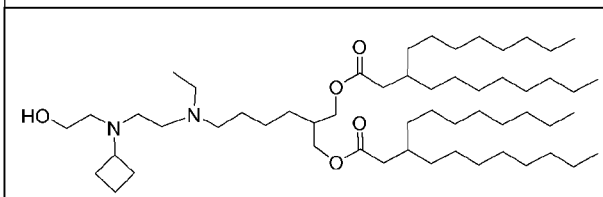
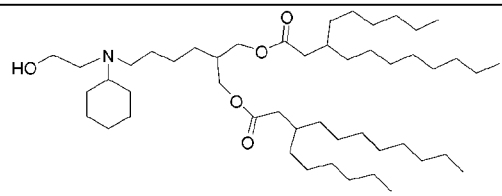
In some embodiments, G^3 is C_2-C_4 alkylene. In some embodiments, G^4 is C_2-C_4 alkylene.

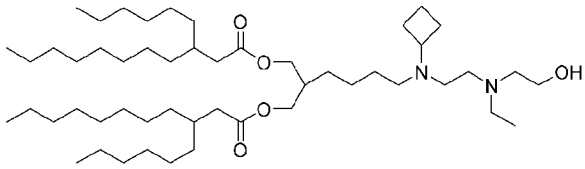
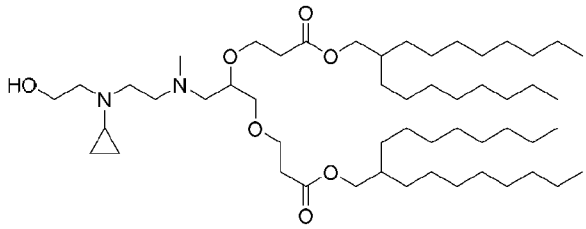
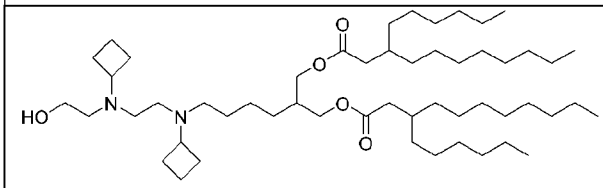
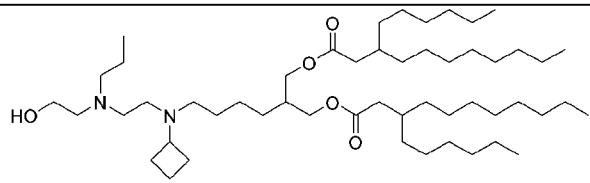
In some embodiments, R^0 is C_1-C_6 alkyl. In some embodiments, R^3 is -OH. In some embodiments, R^3 is $-N(R^4)R^5$. In some embodiments, R^4 is C_3-C_8 cycloalkyl. In some embodiments, R^4 is unsubstituted. In some embodiments, R^5 is $-CH_2CH_2OH$.

In some embodiments, L^1 is $–OC(=O)R^1$, $-C(=O)OR^1$, $-C(=O)R^1$, $-C(=O)NR^bR^c$, or R^1; and L^2 is $–OC(=O)R^2$, $-C(=O)OR^2$, $-C(=O)R^2$, $-C(=O)NR^eR^f$, or R^2. In some embodiments, R^1 and R^2 are each independently branched C_6-C_{24} alkyl or branched C_6-C_{24} alkenyl. In some embodiments, R^1 and R^2 are each independently $-R^7-CH(R^8)(R^9)$, wherein R^7 is C_1-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl or C_2-C_{10} alkenyl. In some embodiments, R^1 is straight C_6-C_{24} alkyl and R^2 is branched C_6-C_{24} alkyl. In some embodiments, R^1 is straight C_6-C_{24} alkyl and R^2 is $-R^7-CH(R^8)(R^9)$, wherein R^7 is C_1-C_5 alkylene, and R^8 and R^9 are independently C_2-C_{10} alkyl.

In some embodiments, the cationic lipid is a compound in Table 4, or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

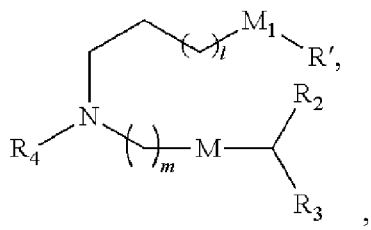
Table 4.

 Compound 04-1	 Compound 04-2
 Compound 04-7	 Compound 04-8
 Compound 04-65	 Compound 04-66
 Compound 04-67	 Compound 04-68

 Compound 04-69	 Compound 04-70
 Compound 04-79	 Compound 04-80

In some embodiments, the cationic lipid contained in the particles or compositions provided herein is a cationic lipid described in U.S. Patent Nos. US10442756B2, US9868691B2, or US9868692B2, all of which are incorporated herein by reference.

In some embodiments, the cationic lipid is a compound Formula (05-I):



(05-I)

or a salt or isomer thereof, wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

M_1 is a bond or M';

R^4 is unsubstituted C_1-C_3 alkyl, or $-(CH_2)_nOH$, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, or heteroaryl, and each n is selected from 1, 2, 3, 4, or 5;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and

R_2 and R_3 are both C_1-C_{14} alkyl, or C_2-C_{14} alkenyl,

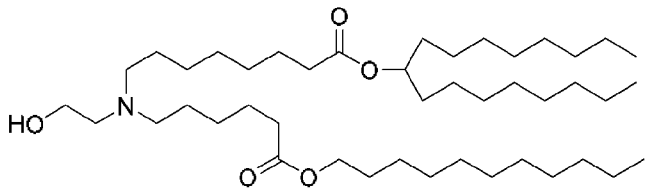
R_8 is selected from the group consisting of C_3-C_6 carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_1-C_6 alkyl, -OR, -$S(O)_2R$, -$S(O)_2N(R)_2$, C_2-C_6 alkenyl, C_3-C_6 carbocycle and heterocycle;

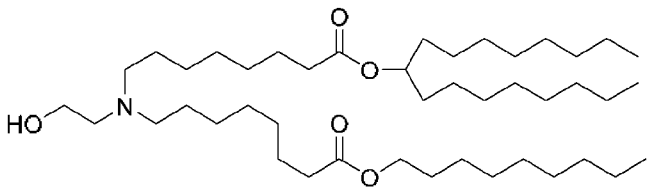
each R is independently selected from the group consisting of C_1-C_3 alkyl, C_2-C_3 alkenyl, and H; and

R' is a linear alkyl.

In some embodiments, the cationic lipid is SM102 or Lipid 5:



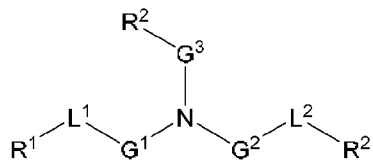
SM102



Lipid 5

In some embodiments, the cationic lipid is a cationic lipid described in U.S. Patent No. US10166298B2, the entire teachings of which are incorporated herein by reference.

In some embodiments, the cationic lipid is a compound of Formula (06-I):



(06-I)

or a pharmaceutically acceptable salt, tautomer, prodrug, or stereoisomer thereof, wherein:

one of L^1 or L^2 is -O(C=O)-, -(C=O)O-, -C(=O)-, -O-, -S(O)$_x$-, -S-S-, -C(=O)S-, SC(=O)-, -NR^{a}C(=O)-, -C(=O)NR^a-, NR^{a}C(=O)NR^a-, -OC(=O)NR^a- or -NR^{a}C(=O)O-, and the other of L^1 or L^2 is -O(C=O)-, -(C=O)O-, -C(=O)-, -O-, -S(O)$_x$-, -S-S-, -C(=O)S-, SC(=O)-, -NR^{a}C(=O)-, -C(=O)NR^a-, NR^{a}C(=O)NR^a-, -OC(=O)NR^a- or -NR^{a}C(=O)O-, or a direct bond;

G^1 and G^2 are each independently unsubstituted C_1-C_{12} alkylene or C_1-C_{12} alkenylene;

G^3 is C_1-C_{24} alkylene, C_1-C_{24} alkenylene, C_3-C_8 cycloalkylene, C_3-C_8 cycloalkenylene;

R^a is H or C_1-C_{12} alkyl;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^3 is H, OR^5, CN, -C(=O)OR^4, -OC(=O)R^4, or -NR^{5}C(=O)R^4;

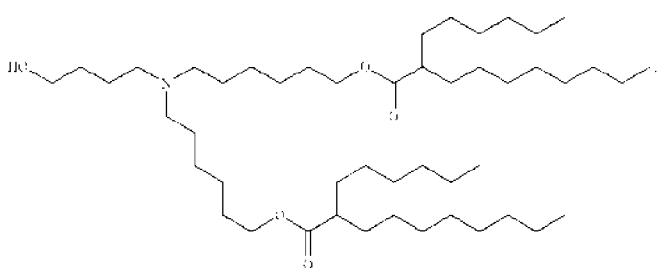
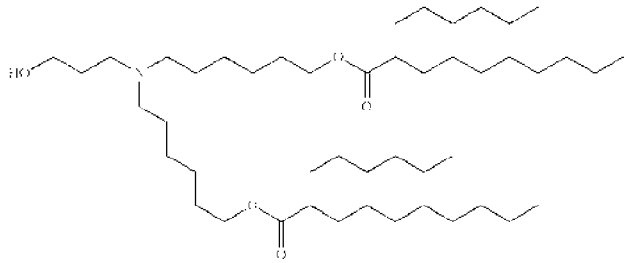
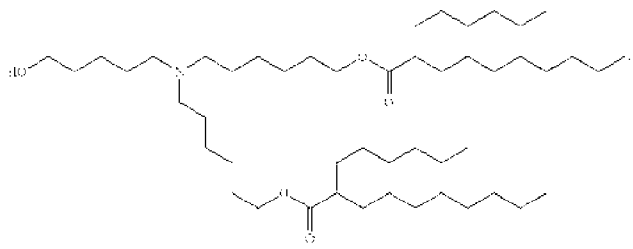
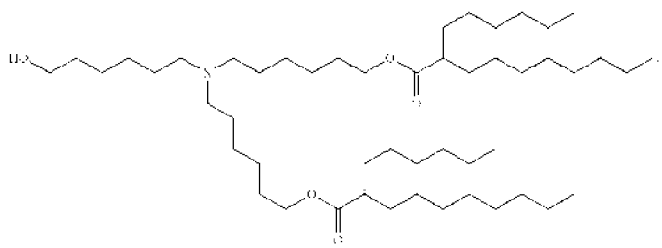
R^4 is C_1-C_{12} alkyl;

R^5 is H or C_1-C_6 alkyl; and

x is 0, 1, or 2.

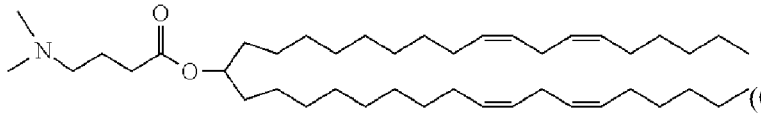
In some embodiments, the cationic lipid is a compound of Table 5, or a pharmaceutically acceptable salt, prodrug, or stereoisomer thereof.

Table 5.

 Compound 06-1	 Compound 06-2
 Compound 06-3	 Compound 06-4

In some embodiments, the cationic lipids of the present disclosure are the same as those disclosed in International Application Publication No. WO 2010/144740, the entire teachings of

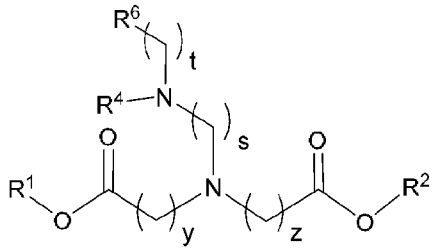
which are incorporated herein by reference. For example, the cationic lipid is a compound represented by Formula (07-I), also named as compound 07-I:



(07-I).

Preferably, the ionizable lipid used in the LNPs according to the present invention is selected from

compounds of Formula (01-I-O):



(01-I-O),

wherein y and z are each independently an integer from 4 to 6,

s is an integer from 2 to 4,

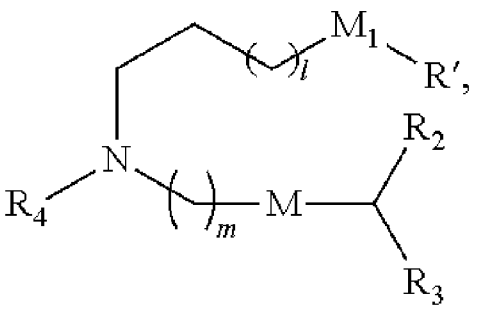
t is an integer from 1 to 3, and

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^4 is C_3-C_8 cycloalkyl;

R^6 is hydrogen or hydroxyl,

compounds of Formula 05-I:



wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

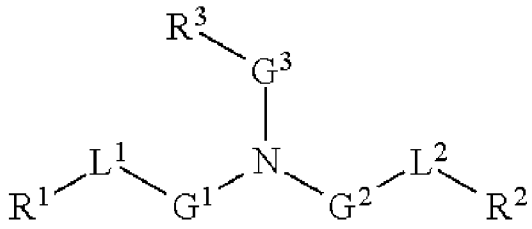
M_1 is -C(O)O-;

R_4 is $-(CH_2)_nOH$, and n is selected from 1, 2, 3, 4, or 5;

M is -OC(O)-; and

R_2 and R_3 are both C_{6-10} alkyl,

compounds of Formula (06-I):



(06-I)

wherein

L^1 and L^2 is -O(C=O)-;

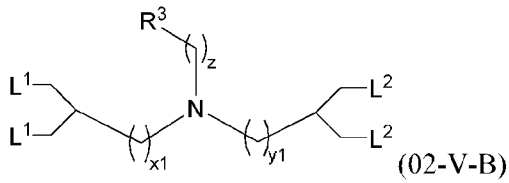
G^1 and G^2 are each independently unsubstituted C_4-C_8 alkylene;

G^3 is C_3-C_8 alkylene;

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^3 is H or OH,

compounds of Formula (02-V-B)



(02-V-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alky;

R^3 is -OR^6;

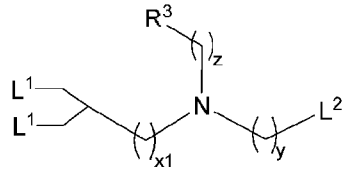
R^6 is hydrogen;

z is an integer from 2 to 12;

x1 is an integer from 0 to 9;

y1 is an integer from 0 to 9;

Compounds of Formula (02-VI-F)



(02-VI-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^3 is -OR^6;

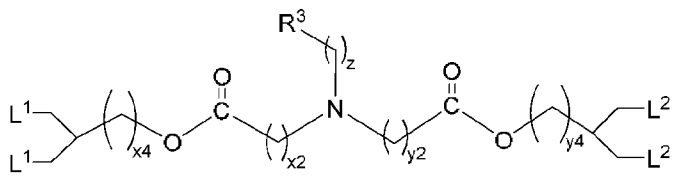
R^6 is hydrogen;

z is an integer from 2 to 12;

y is an integer from 2 to 12;

x1 is an integer from 2 to 5;

compounds of Formula (02-V-F)



(02-V-F)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl;

R^3 is -OR^6;

R^6 is hydrogen;

z is an integer from 2 to 12;

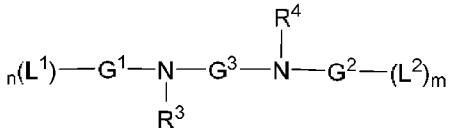
x2 is an integer from 2 to 9;

x4 is an integer from 0 to 3;

y2 is an integer from 2 to 9;

y4 is an integer from 0 to 3,

compounds of formula (03-I)



(03-I),

wherein

G^1 and G^2 are each independently C_3-C_8 alkylene;

each L^1 is independently –OC(=O)R^1 or -C(=O)OR^1;

each L^2 is independently -C(=O)OR^2 or –OC(=O)R^2;

R^1 is independently C_6-C_{24} alkyl;

R^2 is independently C_6-C_{24} alkyl;

G^3 is C_2-C_{12} alkylene;

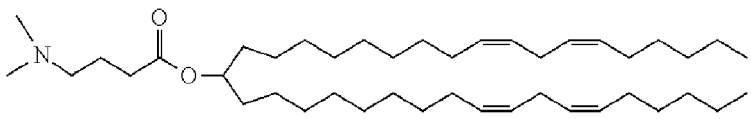
R^3 is C_3-C_8 cycloalkyl;

R^4 is C_1-C_4 hydroxylalkyl;

n is 1 or 2;

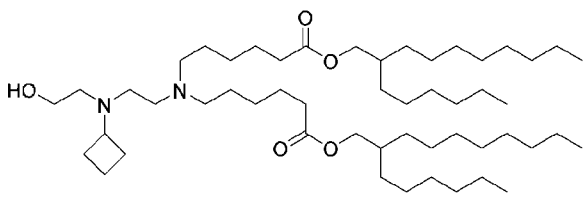
m is 1 or 2, and

compound 07-I:

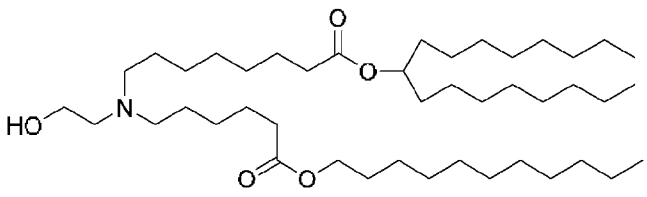


(compound 07-I).

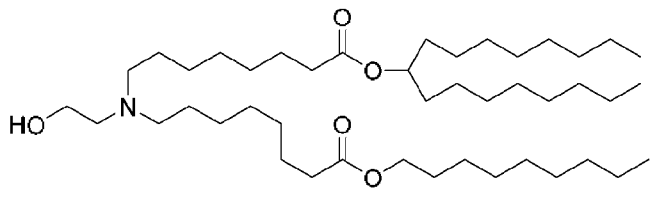
More preferably, the ionizable lipid used in the LNPs according to the present invention is selected from the following compounds:



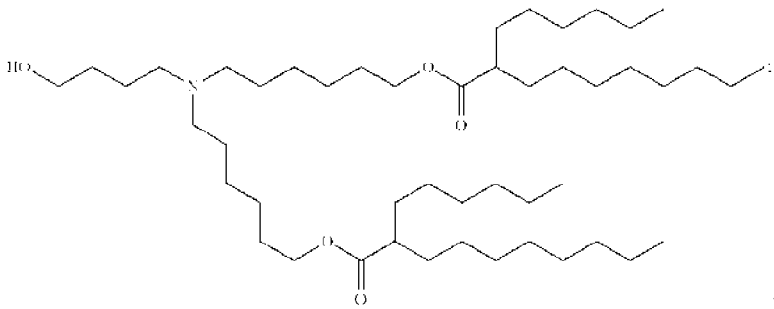
(Compound 01-1)



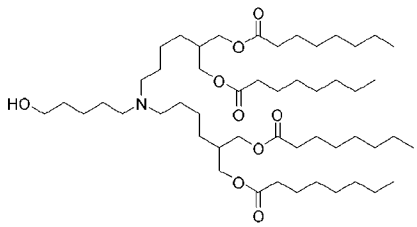
(SM102)



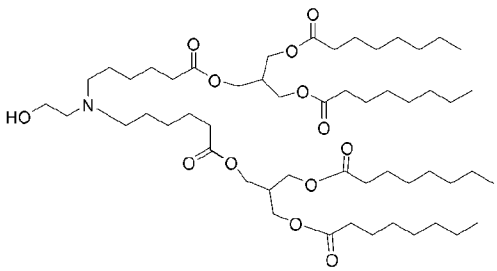
(Lipid 5)



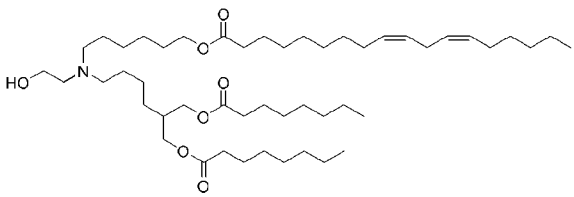
(Compound 06-1)



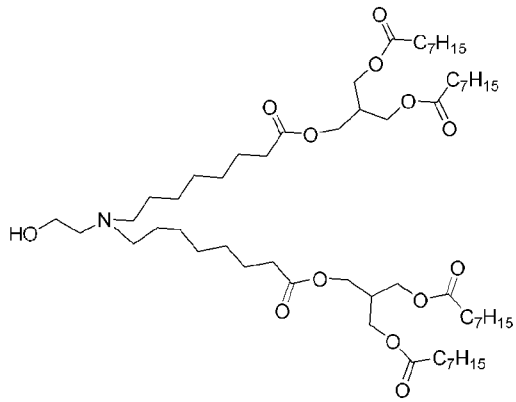
(Compound 02-3)



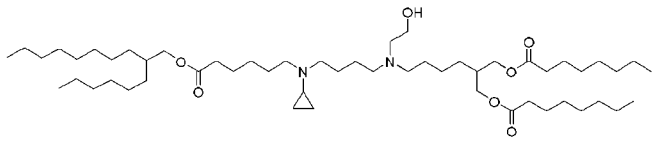
(Compound 02-6)



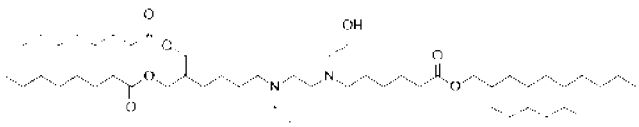
(Compound 02-22)



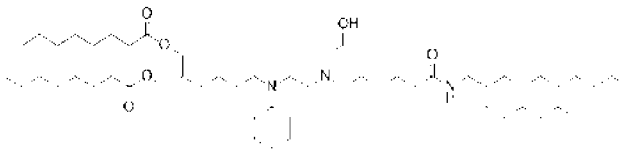
(Compound 02-65)



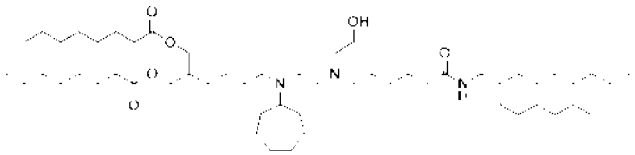
(Compound 03-190)



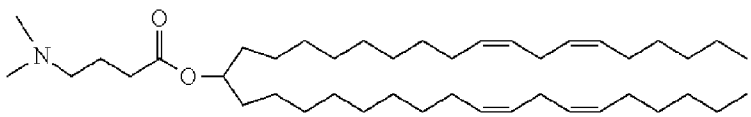
(Compound 03-135)



(Compound 03-140)



(Compound 03-143), and



(compound 07-I).

C. Polymer Conjugated lipids

In some embodiments, the LNP comprises a polymer conjugated lipid. Polymers that can be incorporated into polymer conjugated lipids include polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer

may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellu lose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, polyoxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, or polyvinylpyrrolidone.

In some embodiments, the polymer conjugated lipid is a PEGylated lipid (PEG lipids). Without being bound by the theory, it is contemplated that a polymer conjugated lipid component in an LNP can improve colloidal stability and/or reduce protein absorption of the nanoparticles. Exemplary PEGylated lipids that can be used in connection with the present disclosure include but are not limited to PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. In some embodiments, the PEG-conjugated lipid may be a PEG-modifiedphosphatidylethanolamine,

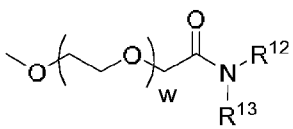
PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, or a PEG-modified dialkylglycerol. In some embodiments, the PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE, Ceramide-PEG2000, or Chol-PEG2000.

In some embodiments, the PEGylated lipid is a PEGylated diacylglycerol (PEG-DAG) such as 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), a pegylated phosphatidylethanoloamine (PEG-PE), a PEG succinate diacylglycerol (PEG-S-DAG) such as 4-O-(2',3'-di(tetradecanoyloxy)propyl-1-O-(ω-methoxy(polyethoxy)ethyl)butanedioate (PEG-S-DMG), a pegylated ceramide (PEG-cer), or a PEG dialkoxypropylcarbamate such as ω-methoxy(polyethoxy)ethyl-N-(2,3-di(tetradecanoxy)propyl)carbamate or 2,3-di(tetradecanoxy)propyl-N-(ω-methoxy(polyethoxy)ethyl)carbamate.

In some embodiments, the PEGylated lipid is present in a concentration ranging from 1.0 to 2.5 molar percent. In some embodiments, the polymer conjugated lipid is present in a concentration of about 1.7 molar percent. In some embodiments, the polymer conjugated lipid is present in a concentration of about 1.5 molar percent.

In some embodiments, the molar ratio of the ionizable lipid to the polymer conjugated lipid ranges from about 20:1 to about 100:1. In some embodiments, the molar ratio of the ionizable lipid to polymer conjugated lipid ranges from about 25:1 to about 80:1.In some embodiments, the molar ratio of the ionizable lipid to polymer conjugated lipid ranges from about 30:1 to about 60:1. In some embodiments, the molar ratio of the ionizable lipid to polymer conjugated lipid ranges from about 30:1 to about 50:1.

In some embodiments, the PEGylated lipid has the following Formula:



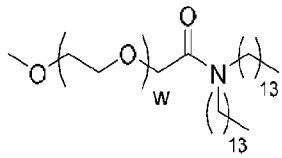
or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, wherein:

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w has a mean value ranging from 30 to 60.

In some embodiments, R^{12} and R^{13} are each independently straight, saturated alkyl chains containing from 12 to 16 carbon atoms. In other embodiments, the average w ranges from 42 to 55, for example, the average w is 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54 or 55. In some embodiments, the average w is about 49.

In some embodiments, the PEGylated lipid has the following Formula:



or a pharmaceutically salt thereof, wherein the average w is about 49.

D. Lipid stabilizer

In some embodiments, the LNPs comprise a lipid stabilizer. In some embodiments, the lipid stabilizer comprises a sterol. In some embodiments, the lipid stabilizer comprises a corticosteroid. In some embodiments, the lipid stabilizer comprises two or more components. In some embodiments, the lipid stabilizer comprises a corticosteroid and a sterol. In some embodiments, the sterol is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, and brassicasterol. In some embodiments, the corticosteroid is selected from the group consisting of prednisolone, dexamethasone, prednisone, and hydrocortisone. In some embodiments, the lipid stabilizer comprises tomatidine, tomatine, ursolic acid, or alpha-tocopherol. In some embodiments, the lipid stabilizer comprises one or more compounds selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, prednisolone, dexamethasone, prednisone, hydrocortisone, tomatidine, tomatine, ursolic acid, and alpha-tocopherol. In some embodiments, the lipid stabilizer is cholesterol.

E. LNPs comprising mRNA

In some embodiments, the LNPs disclosed herein further comprise a therapeutic payload. The payload can be any substance or compound that has a therapeutic or prophylactic effect. In some embodiments, the therapeutic payload is a small molecule, a cytotoxin, a radioactive ion, a chemotherapeutic compound, a vaccine, or a compound that elicits an immune response.

In some embodiments, the LNPs disclosed herein comprise a nucleic acid. In some embodiments, the nucleic acid is a DNA. In some embodiments, the DNA is catalytic DNA, plasmid DNA, aptamer, or complementary DNA (cDNA). In some embodiments, the nucleic

acid is an RNA. In some embodiments, the RNA is a messenger RNA (mRNA), antisense oligonucleotide, microRNA (miRNA), miRNA inhibitor (*e.g.*, antagomir or antimir), messenger-RNA-interfering complementary RNA (micRNA), multivalent RNA, dicer substrate RNA (dsRNA), small hairpin RNA (shRNA), antisense RNA, transfer RNA (tRNA), asymmetrical interfering RNA (aiRNA), a ribozyme, an aptamer, or a vector. In some embodiments, the RNA is an mRNA hybrid. In some embodiments, the nucleic acid is an mRNA. In some embodiments, the mRNA encodes a protein. In some embodiments, the protein is an antibody. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the LNPs comprise an RNAi agent or RNAi-inducing agent. Preferably, the weight ratio of the ionizable lipid to the therapeutic payload is from 5:1 to 20:1.

F. Compositions comprising Lipid Nanoparticles (LNPs)

The present disclosure is inclusive with respect to compositions comprising the LNPs described herein. In some embodiments, the composition comprises a plurality of LNPs. In some embodiments, the plurality of LNPs have a polydispersity index (PDI) of 0.001 to 0.2. In some embodiments, the plurality of LNPs have a polydispersity index (PDI) of 0.001 to 0.1. In some embodiments, the LNPs have a PDI of 0.005 to 0.05.

In some embodiments, some of the LNPs of the LNP composition comprise mRNA. In some embodiments, the mRNA encapsulation efficiency (EE% - *i.e.*, the percentage of individual LNPs in the composition that encapsulate the mRNA) of the LNP composition is from 70% to 100%. In some embodiments, the EE% of the LNP composition is from 80 to 95%. In some embodiments, the EE% of the LNP composition is from 85 to 95%. In some embodiments, the EE% of the LNP composition is from 90 to 95%. In some embodiments, the EE% is 80% or greater. In some embodiments, the EE% is 85% or greater. In some embodiments, the EE% is 90% or greater. In some embodiments, the EE% is 95% or greater. In some embodiments, the EE% is 80%-100%. In some embodiments, the EE% is 85%-100%. In some embodiments, the EE% is 90%-100%. In some embodiments, the EE% is 95%-100%.

In some embodiments, the LNP composition is able to increase protein expression compared to comparable LNP compositions that do not comprise a steroid-containing phospholipid.

It is to be understood that any of the molar percentages or molar ratios described above for the sterol-containing phospholipid, the ionizable lipid, the polymer conjugated lipid, and the lipid

stabilizer may be combined with each other in any embodiment describing the composition of LNPs described herein, such that every combination is contemplated as though each and every combination were specifically and individually disclosed.

G. Methods of Making the LNPs and compositions thereof

The LNPs here can be made according to the methods that are well known in the art.

In some embodiments, the method comprises solubilizing the lipid components (*e.g.*, a phospholipid, an ionizable lipid, a polymer conjugated lipid, and a lipid stabilizer) in a solvent. In some embodiments, the method comprises the steps:

(a) solubilizing the lipid components (*e.g.*, a phospholipid, an ionizable lipid, a polymer conjugated lipid, and optionally a lipid stabilizer) in a solvent to produce a lipid mixture;

(b) diluting the mRNA in a solvent to produce an mRNA mixture; and

(c) mixing the lipid mixture and mRNA mixture to obtain an mRNA LNP mixture.

In some embodiments, the solvent in step (a) is a polar solvent. In some embodiments, the solvent in step (a) is an alcohol solvent. In some embodiments, the solvent in step (a) is methanol, ethanol, n-propanol, or isopropanol. In some embodiments, the solvent in step (a) is ethanol.

In some embodiments, the solvent in step (b) is an aqueous solvent. In some embodiments, the solvent in step (b) is an aqueous buffer. In some embodiments, the solvent in step (b) is a citrate buffer. In some embodiments, the citrate buffer has a citrate concentration of 5 to 100 mM. In some embodiments, the citrate buffer has a citrate concentration of 10 to 50 mM. In some embodiments, the aqueous solvent of step (b) has a pH of 2-6. In some embodiments, the aqueous solvent of step (b) has a pH of 3-5.

In some embodiments, the mixing of step (c) occurs with a weight ratio of lipid:mRNA of 10:1 to 30:1. In some embodiments, the mixing of step (c) occurs at a volume ratio of lipid:mRNA of 1:1 to 1:5. In some embodiments, the volume ratio is 1:2 to 1:4. In some embodiments, the volume ratio is about 1:3. In some embodiments, the mixing of step (c) is performed with a microfluidic apparatus. In some embodiments, the microfluidic apparatus has a flow rate of 9 to 30 mL/min.

In some embodiments, the mRNA mixture has an mRNA concentration from 1 to 3, 5, 7, 10, 12, 15, 20, 30, 40 or 50 mM. In some embodiments, the concentration is from 3 to 5, 7, 10, 12, 15, 20, or 30mM. In some embodiments, the concentration is from 5 to 7, 10, 12, 15, 20, or

30 mM. In some embodiments, the concentration is from 7 to 10, 12, 15, 20, or 30 mM. In some embodiments, the concentration is from 10 to 12, 15, 20, or 30 mM. In some embodiments, the concentration is from 12 to 15, 20, or 30 mM. In some embodiments, the concentration is from 15 to 20 or 30 mM. In some embodiments, the concentration is from 20 to 30 mM. In some embodiments, the concentration is about 5, 7, 10, 12, 15, or 20 mM. In some embodiments, the concentration is about 5, 7, 10, 12, or 15 mM. In some embodiments, the concentration is about 10 mM. In some embodiments, the concentration is about 12 mM. In some embodiments, the concentration is about 15 mM. In some embodiments, the concentration is about 20 mM.

In some embodiments, the method further comprises a step (d):

(d) filtering the lipid nanoparticle through a sterile filter.

In some embodiments, the sterile filter is a 0.2 μm sterile filter.

IV. Methods Related to the Lipid Nanoparticles (LNPs)

The present disclosure is inclusive with respect to potentially useful methods that make use of the LNPs described herein.

In some embodiments, a method for expressing protein in a cell, wherein the method comprises introducing an LNP or composition thereof, as described above, to a cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the LNP or composition thereof is administered systemically to a mammal. In some embodiments, the mammal is a human.

In some embodiments, the LNP composition is able to increase protein expression compared to comparable LNP compositions that do not comprise a steroid-containing phospholipid.

V. Kits comprising a phospholipid containing a sterol moiety

The present disclosure includes kits comprising a phospholipid containing a sterol moiety and packaging for said phospholipid. In some embodiments, the kit further comprises an ionizable lipid. In some embodiments, the kit further comprises a polymer conjugated lipid. In some embodiments, the kit further comprises a lipid stabilizer. In some embodiments, the kit further comprises an ionizable lipid, a polymer conjugated lipid, and a lipid stabilizer. In some embodiments, the ionizable lipid is a cationic lipid. In some embodiments, the polymer conjugated lipid is a PEGylated lipid. In some embodiments, the lipid stabilizer is cholesterol. In some embodiments, the kit further comprises a cationic lipid, a PEGylated lipid, and cholesterol.

It is understood that any embodiment of the compounds provided herein, as set forth above, and any specific substituent and/or variable in the compound provided herein, as set forth above, may be independently combined with other embodiments and/or substituents and/or variables of the compounds to form embodiments not specifically set forth above. In addition, in the event that a list of substituents and/or variables is listed for any particular group or variable, it is understood that each individual substituent and/or variable may be deleted from the particular embodiment and/or claim and that the remaining list of substituents and/or variables will be considered to be within the scope of embodiments provided herein.

It is understood that in the present description, combinations of substituents and/or variables of the depicted formulae are permissible only if such contributions result in stable compounds.

EXEMPLARY EMBODIMENTS

The following exemplary embodiments are provided herein:

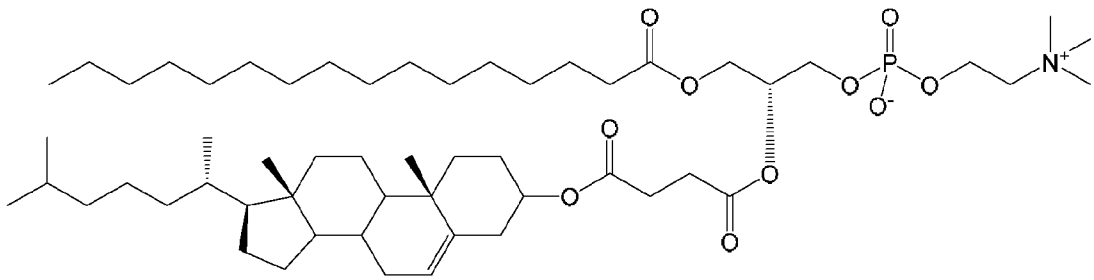
Embodiment 1. A lipid nanoparticle (LNP) comprising

a phospholipid containing a sterol moiety;

an ionizable lipid; and

a polymer conjugated lipid.

Embodiment 2. The LNP of embodiment 1, wherein the phospholipid has a structure selected from:



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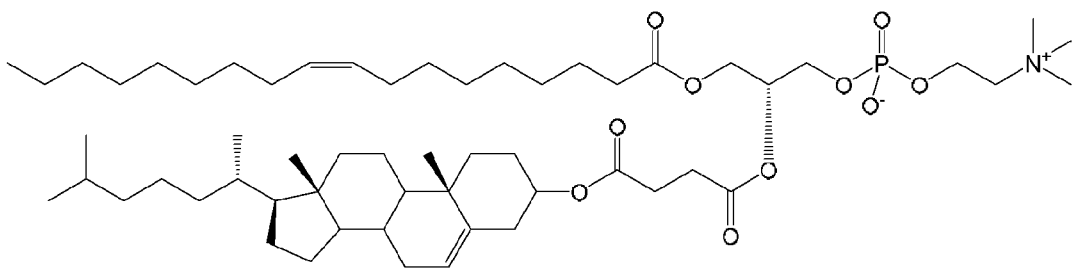
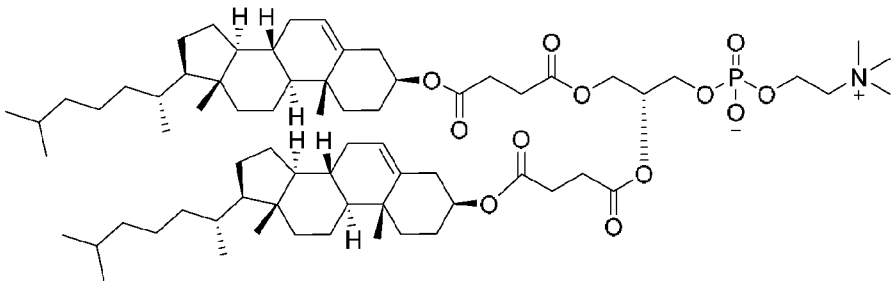
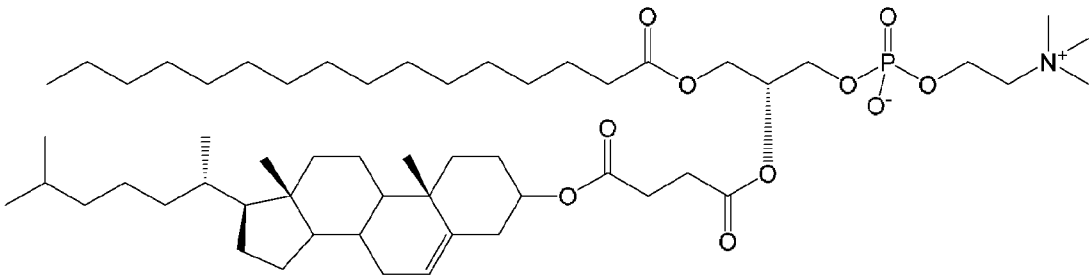


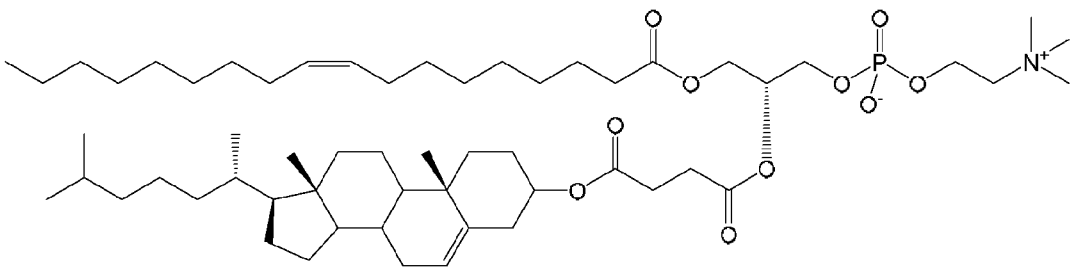
, and



Embodiment 3. The LNP of embodiment 1 or 2, wherein the phospholipid has the structure:



Embodiment 4. The LNP of embodiment 1 or 2, wherein the phospholipid has the structure:



Embodiment 5. The LNP of any one of embodiments 1 to 4, wherein the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1.

Embodiment 6. The LNP of embodiment 5, wherein the molar ratio of the ionizable lipid to the phospholipid is from 15:1 to 5:1.

Embodiment 7. The LNP of any one of embodiments 1 to 4, wherein the ionizable lipid comprises from 40 to 80 mol% of a total amount of lipids in the LNP.

Embodiment 8. The LNP of embodiment 7, wherein the ionizable lipid comprises from 50 to 70 mol% of the total amount of lipids in the LNP.

Embodiment 9. The LNP of any one of embodiments 1 to 8, wherein the ionizable lipid is a cationic lipid.

Embodiment 10. The LNP of any one of embodiments 1 to 8, wherein the ionizable lipid is a compound according to any one of the formula selected from 01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, or wherein the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5.

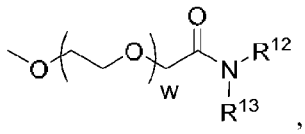
Embodiment 11. The LNP of any one of embodiments 1 to 10, wherein the polymer conjugated lipid comprises from 1 to 2% of a total amount of lipids in the LNP.

Embodiment 12. The LNP of embodiment 11, wherein the polymer conjugated lipid comprises 1.5% of the total amount of lipids in the LNP.

Embodiment 13. The LNP of any one of embodiments 1 to 12, wherein the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid from 1:5 to 1:10.

Embodiment 14. The LNP of any one of embodiments 1 to 13, wherein the polymer conjugated lipid is a PEGylated lipid.

Embodiment 15. The LNP of any one of embodiments 1 to 13, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



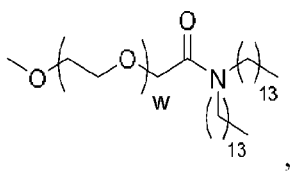
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60.

Embodiment 16. The LNP of any one of embodiments 1 to 15, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



,

or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60.

Embodiment 17. The LNP of embodiment 15 or 16, wherein w is an integer ranging from 45 to 55.

Embodiment 18. The LNP of embodiment 15 or 16, wherein w is about 49.

Embodiment 19. The LNP of any one of embodiments 1 to 14, wherein the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

Embodiment 20. The LNP of any one of embodiments 1 to 19, further comprising a lipid stabilizer.

Embodiment 21. The LNP of embodiment 20, wherein the LNP has a molar ratio of the lipid stabilizer to the phospholipid from 10:1 to 1:4.

Embodiment 22. The LNP of embodiment 21, wherein the molar ratio of the lipid stabilizer to the phospholipid is from 5:1 to 1:1.

Embodiment 23. The LNP of embodiment 21, wherein the molar ratio of the lipid stabilizer to the phospholipid is from 4:1 to 3:1.

Embodiment 24. The LNP of any one of embodiments 20 to 23, wherein the lipid stabilizer comprises from 5 to 50 mol% of a total amount of lipids in the LNP.

Embodiment 25. The LNP of embodiment 24, wherein the lipid stabilizer comprises from 8 to 40 mol% of the total amount of lipids in the LNP.

Embodiment 26. The LNP of embodiment 24, wherein the lipid stabilizer comprises from 10 to 30 mol% of the total amount of lipids in the LNP.

Embodiment 27. The LNP of any one of embodiments 1 to 26, wherein the phospholipid comprises from 1 to 30 mol% of a total amount of lipids in the LNP.

Embodiment 28. The LNP of embodiment 27, wherein the phospholipid comprises from 2 to 25 mol% of the total amount of lipids in the LNP.

Embodiment 29. The LNP of embodiment 27, wherein the phospholipid comprises from 3 to 20 mol% of the total amount of lipids in the LNP.

Embodiment 30. The LNP of embodiment 27, wherein the phospholipid comprises from 5 to 15 mol% of the total amount of lipids in the LNP.

Embodiment 31. The LNP of embodiment 27, wherein the phospholipid comprises about 10 mol% of the total amount of lipids in the LNP.

Embodiment 32. The LNP of any one of embodiments 1 to 31, wherein the LNP has a size from 50 nm to 150 nm, as determined using dynamic light scattering.

Embodiment 33. The LNP of embodiment 32, wherein the size is from 60 nm to 140 nm.

Embodiment 34. The LNP of embodiment 32, wherein the size is from 80 nm to 100 nm.

Embodiment 35. The LNP of embodiment 32, wherein the size is from 85 nm to 95 nm.

Embodiment 36. The LNP of any one of embodiments 1 to 35, wherein the LNP encapsulates mRNA.

Embodiment 37. A composition comprising lipid nanoparticles (LNPs), wherein each LNP is an LNP of any one of embodiments 1 to 36.

Embodiment 38. The composition of embodiment 37, wherein at least 80% of the LNPs encapsulate mRNA.

Embodiment 39. The composition of embodiment 37, wherein at least 85% of the LNPs encapsulate mRNA.

Embodiment 40. A method for expressing protein in a cell, comprising introducing the LNP of embodiment 36 or the composition of embodiment 38 or 39, to the cell.

Embodiment 41. The method of embodiment 40, wherein the cell is a mammalian cell.

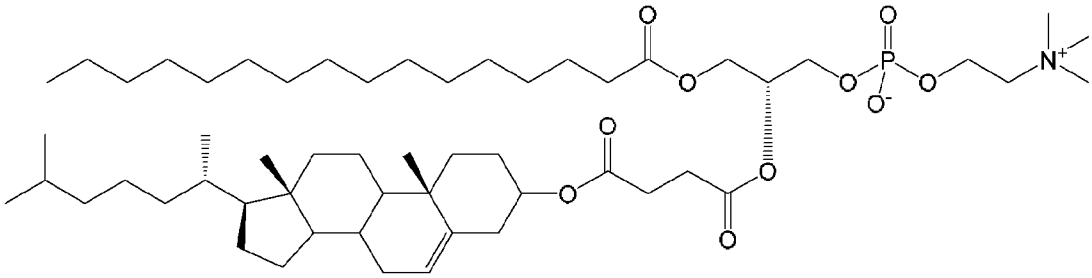
Embodiment 42. A method for delivering a protein to a subject, comprising administering the LNP of embodiment 36 or the composition of embodiment 38 or 39 to the individual, wherein the mRNA encodes the protein.

Embodiment 43. The method of embodiment 42, wherein the LNP or the composition is administered systemically.

Embodiment 44. The method of embodiment 42, wherein the subject is a mammal.

Embodiment 45. The method of embodiment 42, wherein the subject is a human.

Embodiment 46. A lipid nanoparticle (LNP) comprising a phospholipid, wherein the phospholipid has a structure selected from:



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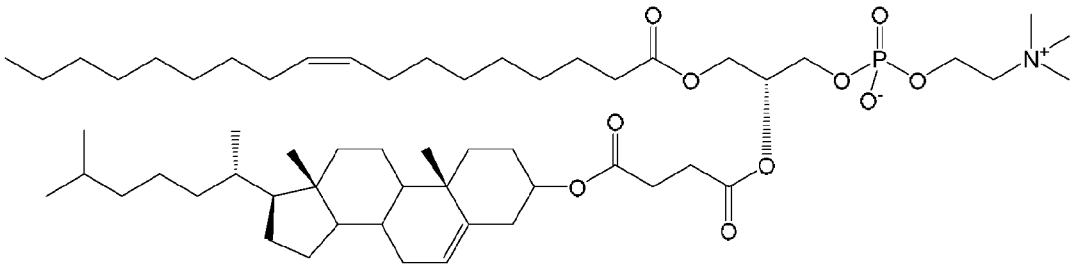
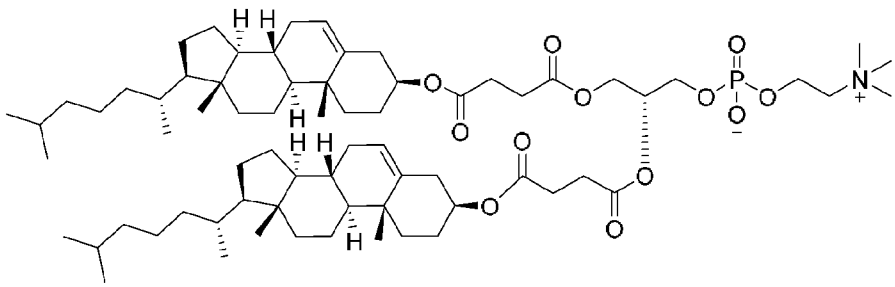
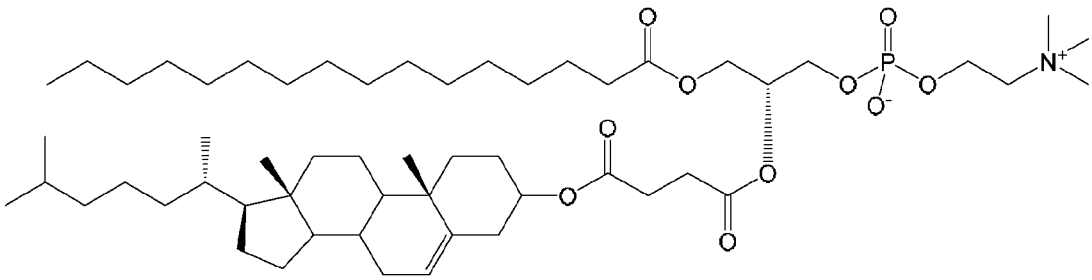


, and



.

Embodiment 47. The LNP of embodiment 46, wherein the phospholipid has the structure:



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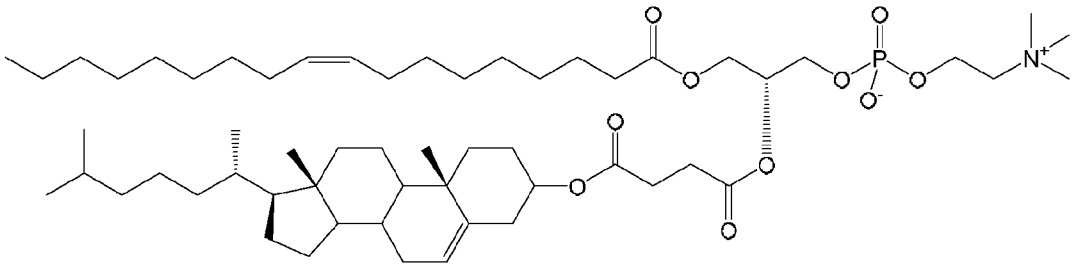
Embodiment 48. The LNP of embodiment 46, wherein the phospholipid has the structure:



.

Embodiment 49. The LNP of any one of embodiments 46-48, further comprising an ionizable lipid.

Embodiment 50. The LNP of embodiment 49, wherein the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1.

Embodiment 51. The LNP of embodiment 50, wherein the molar ratio of ionizable lipid to phospholipid is from 15:1 to 5:1.

Embodiment 52. The LNP of embodiment 49, wherein the ionizable lipid comprises from 40 to 80 mol% of a total amount of lipids in the LNP.

Embodiment 53. The LNP of embodiment 52, wherein the ionizable lipid comprises from 50 to 70 mol% of the total amount of lipids in the LNP.

Embodiment 54. The LNP of any one of embodiments 49 to 53, wherein the ionizable lipid is a compound according to any one of the formula selected from 01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, or wherein the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5.

Embodiment 55. The LNP of any one of embodiments 49 to 54, wherein the ionizable lipid is a cationic lipid.

Embodiment 56. The LNP of any one of embodiments 46 to 55, further comprising a polymer conjugated lipid.

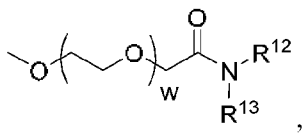
Embodiment 57. The LNP of embodiment 56, wherein the polymer conjugated lipid comprises from 1 to 2% of a total amount of lipids in the LNP.

Embodiment 58. The LNP of embodiment 57, wherein the polymer conjugated lipid comprises 1.5% of the total amount of lipids in the LNP.

Embodiment 59. The LNP of any one of embodiments 46 to 58, wherein the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid from 1:5 to 1:10.

Embodiment 60. The LNP of any one of embodiments 56 to 59, wherein the polymer conjugated lipid is a PEGylated lipid.

Embodiment 61. The LNP of any one of embodiments 56 to 60, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



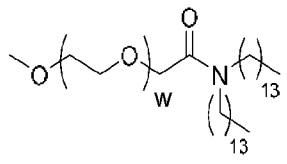
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60.

Embodiment 62. The LNP of any one of embodiments 56 to 61, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



,

or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60.

Embodiment 63. The LNP of embodiment 61 or 62, wherein w is an integer ranging from 45 to 55.

Embodiment 64. The LNP of embodiment 61 or 62, wherein w is about 49.

Embodiment 65. The LNP of any one of embodiments 56 to 60, wherein the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

Embodiment 66. The LNP of any one of embodiments 46 to 65, further comprising a lipid stabilizer.

Embodiment 67. The LNP of embodiment 66, wherein the LNP has a molar ratio of the lipid stabilizer to the phospholipid from 10:1 to 1:4.

Embodiment 68. The LNP of embodiment 67, wherein the molar ratio of the lipid stabilizer to the phospholipid is from 5:1 to 1:1.

Embodiment 69. The LNP of embodiment 67, wherein the molar ratio of the lipid stabilizer to the phospholipid is from 4:1 to 3:1.

Embodiment 70. The LNP of any one of embodiments 66 to 69, wherein the lipid stabilizer comprises from 5 to 50 mol% of a total amount of lipids in the LNP.

Embodiment 71. The LNP of embodiment 70, wherein the lipid stabilizer comprises from 8 to 40 mol% of the total amount of lipids in the LNP.

Embodiment 72. The LNP of embodiment 70, wherein the lipid stabilizer comprises from 10 to 30 mol% of the total amount of lipids in the LNP.

Embodiment 73. The LNP of any one of embodiments 46 to 72, wherein the phospholipid comprises from 1 to 30 mol% of a total amount of lipids in the LNP.

Embodiment 74. The LNP of embodiment 73, wherein the phospholipid comprises from 2 to 25 mol% of the total amount of lipids in the LNP.

Embodiment 75. The LNP of embodiment 73, wherein the phospholipid comprises from 3 to 20 mol% of the total amount of lipids in the LNP.

Embodiment 76. The LNP of embodiment 73, wherein the phospholipid comprises from 5 to 15 mol% of the total amount of lipids in the LNP.

Embodiment 77. The LNP of embodiment 73, wherein the phospholipid comprises about 10 mol% of the total amount of lipids in the LNP.

Embodiment 78. The LNP of any one of embodiments 46 to 77, wherein the LNP has a size from 50 nm to 150 nm, as determined using dynamic light scattering.

Embodiment 79. The LNP of embodiment 78, wherein the size is from 60 nm to 140 nm.

Embodiment 80. The LNP of embodiment 78, wherein the size is from 80 nm to 100 nm.

Embodiment 81. The LNP of embodiment 78, wherein the size is from 85 nm to 95 nm.

Embodiment 82. The LNP of any one of embodiments 46 to 81, wherein the LNP encapsulates mRNA.

Embodiment 83. A composition comprising lipid nanoparticles (LNPs), wherein each LNP is an LNP of any one of embodiments 46 to 82.

Embodiment 84. The composition of embodiment 83, wherein at least 80% of the LNPs encapsulate mRNA.

Embodiment 85. The composition of embodiment 83, wherein at least 85% of the LNPs encapsulate mRNA.

Embodiment 86. A method for expressing protein in a cell, comprising introducing the LNP of embodiment 82 or the composition of embodiment 84 or 85, to the cell.

Embodiment 87. The method of embodiment 86, wherein the cell is a mammalian cell.

Embodiment 88. A method for delivering a protein to a subject, comprising administering the LNP of embodiment 82 or the composition of embodiment 84 or 85 to the individual, wherein the mRNA encodes the protein.

Embodiment 89. The method of embodiment 88, wherein the LNP or the composition is administered systemically.

Embodiment 90. The method of embodiment 88, wherein the subject is a mammal.

Embodiment 91. The method of embodiment 88, wherein the subject is a human.

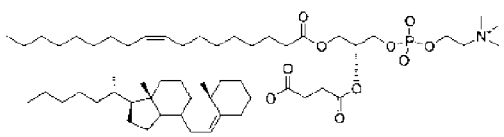
EXAMPLES

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

General preparative High Performance Liquid Chromatography (HPLC) method: HPLC purification is carried out on a Waters 2767 equipped with a diode array detector (DAD) on an Inertsil Pre-C8 OBD column, generally with water containing 0.1% trifluoroacetic acid (TFA) as solvent A and acetonitrile as solvent B.

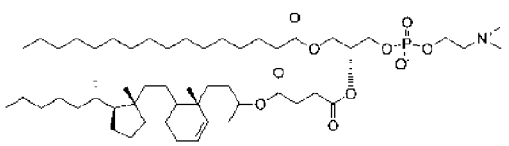
General Liquid Chromatography-Mass Spectrometry (LCMS) method: LCMS analysis is conducted on a Shimadzu (LC-MS2020) System. Chromatography is performed on a SunFire C18, generally with water containing 0.1% formic acid as solvent A and acetonitrile containing 0.1% formic acid as solvent B.

The Abbreviation OChemsPC refers to the following compound:



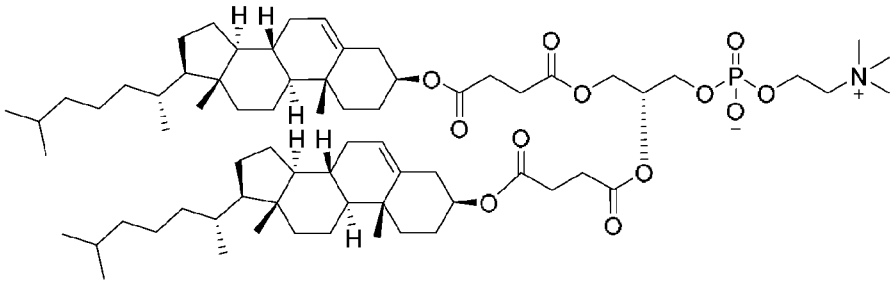
.

The Abbreviation PChemsPC refers to the following compound:



.

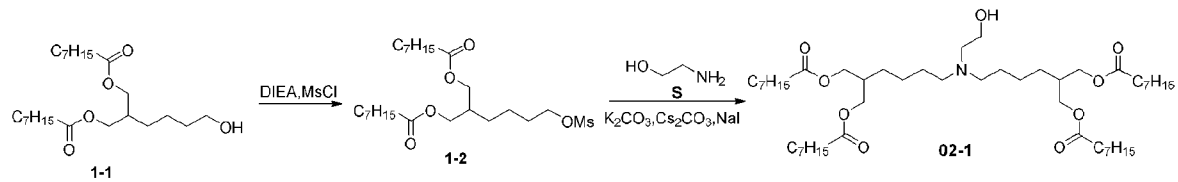
The Abbreviation DChemsPC refers to the following compound:



"DSPC" refers to distearoylphosphatidylcholine. "compound 01-1" refers to compound 01-1 in Table 1. "Chol" is an abbreviation for cholesterol. "DMG-PEG" refers to 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000. ALC-0315 refers to compound 06-1, MC3 refers to compound 07-I.

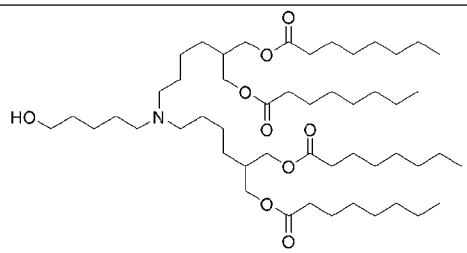
Example 1. Preparation of Compound 02-1 and Compound 02-3

Compound 02-1 was prepared according to the scheme below.



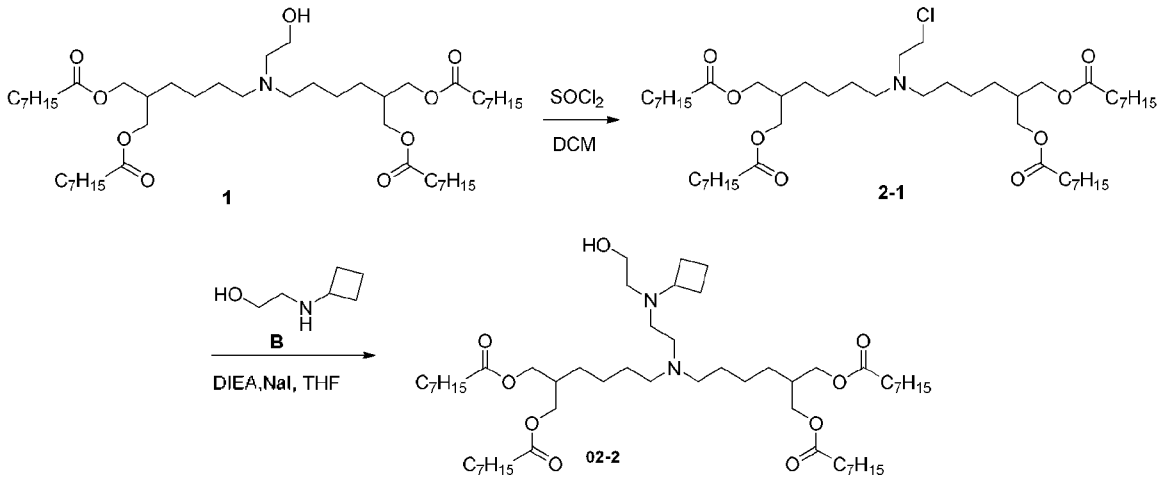
Compound 02-1: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.27-1.63 (m, 53H), 1.97-2.01 (m, 2H), 2.28-2.64 (m, 14H), 3.52-3.58 (m, 2H), 4.00-4.10 (m, 8H). LCMS: Rt: 1.080 min; MS m/z (ESI): 826.0 $[M+H]^+$.

Compound 02-3 was prepared in analogous fashion as Compound 02-1, using corresponding starting material.

Compound	Characterization
 Compound 02-3	^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.28-1.32 (m, 30H), 1.35-1.44 (m, 12H), 1.57-1.66 (m, 17H), 1.95-2.01 (m, 2H), 2.28-2.40 (m, 14H), 3.63-3.66 (m, 2H), 4.00-4.11 (m, 8H). LCMS: Rt: 1.140 min; MS m/z (ESI): 868.1 $[M+H]^+$.

Example 2. Preparation of Compound 02-2

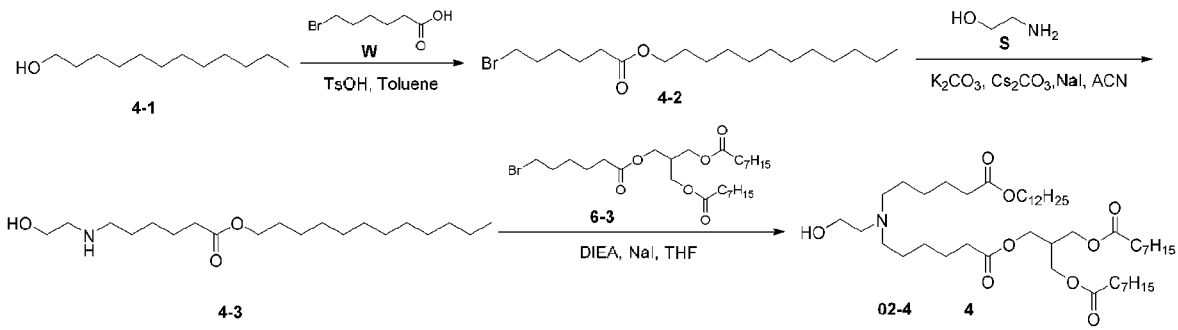
Compound 02-2 was prepared according to the scheme below.



Compound 02-2: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.28-1.67 (m, 54H), 1.88-2.01 (m, 7H), 2.28-2.56 (m, 18H), 3.16-3.20 (m, 1H), 3.52-3.54 (m, 2H), 4.00-4.10 (m, 8H). LCMS: Rt: 1.060 min; MS m/z (ESI): 923.0 $[M+H]^+$.

Example 3. Preparation of Compound 02-4

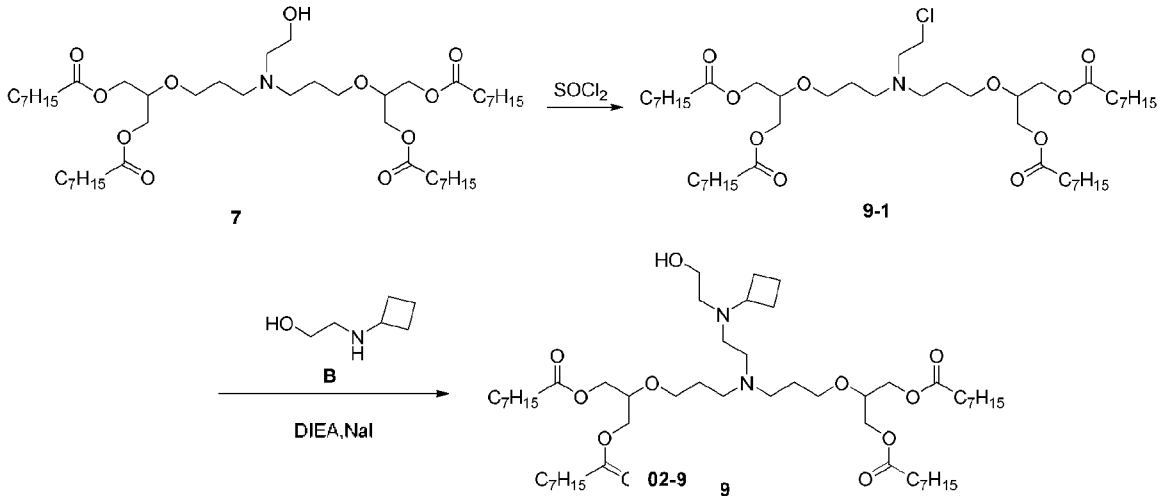
Compound 02-4 was prepared according to the scheme below.



Compound 02-4: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 9H), 1.26-1.32 (m, 34H), 1.41-1.49 (m, 4H), 1.61-1.66 (m, 15H), 2.00-2.03 (m, 1H), 2.21-2.38 (m, 8H), 2.43-2.47 (m, 4H), 2.56-2.60 (m, 2H), 3.50-3.54 (m, 2H), 4.03-4.14 (m, 8H). LCMS: Rt: 1.030 min; MS m/z (ESI): 798.0 $[M+H]^+$.

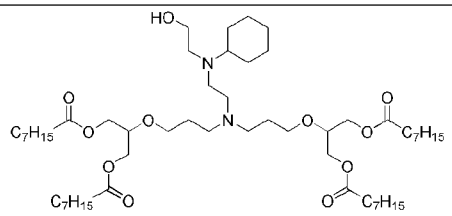
Example 4. Preparation of Compound 02-9 and Compound 02-14

Compound 02-9 was prepared according to the scheme below.



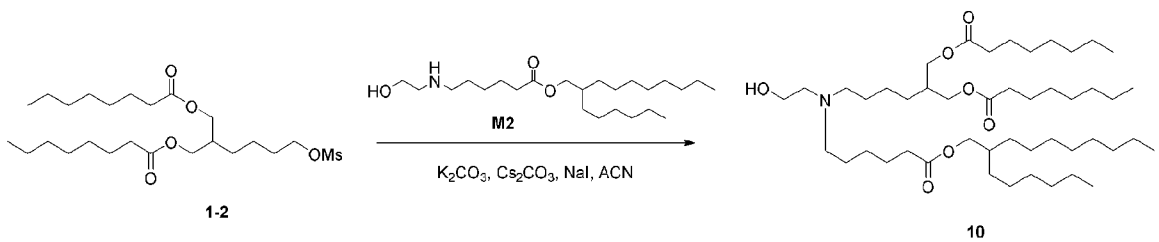
Compound 02-9: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.28-1.30 (m, 33H), 1.58-2.01 (m, 18H), 2.30-2.54 (m, 18H), 3.10-3.19 (m, 1H), 3.52-3.68 (m, 8H), 4.09-4.20 (m, 8H). LCMS: Rt: 1.677 min; MS m/z (ESI): 927.7 $[M+H]^+$.

Compound 02-14 was prepared in analogous fashion as Compound 02-9, using corresponding starting material.

Compound	Characterization
 Compound 02-14	^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.28-1.30 (m, 40H), 1.58-1.80 (m, 16H), 2.30-2.60 (m, 18H), 3.58-3.68 (m, 8H), 4.08-4.20 (m, 8H). LCMS: Rt: 1.000 min; MS m/z (ESI): 955.7 $[M+H]^+$.

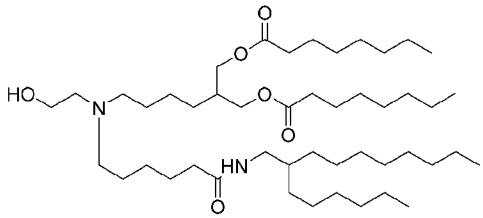
Example 5. Preparation of Compound 02-10 and Compound 02-11

Compound 02-10 was prepared according to the scheme below.



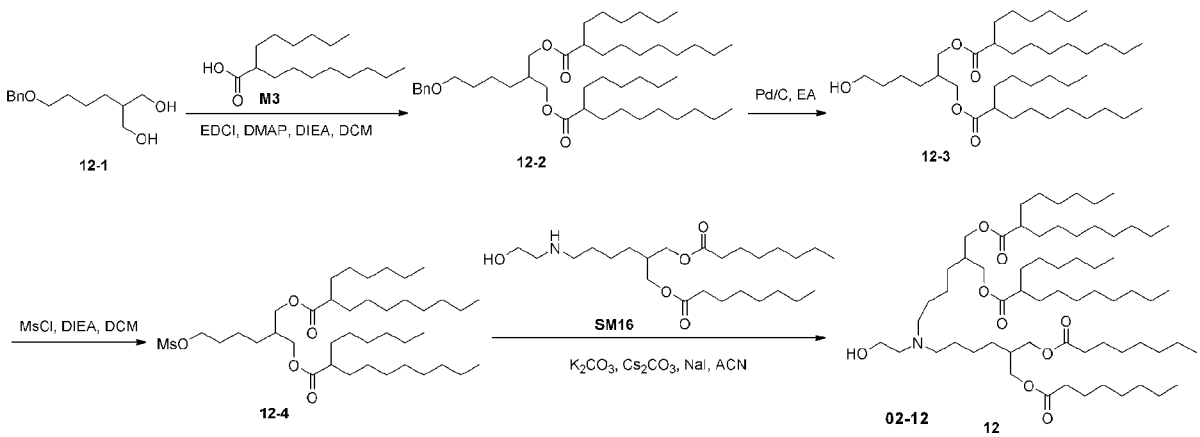
Compound 02-10: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.26-1.41 (m, 48H), 1.51-1.72 (m, 11H), 1.94-2.03 (m, 1H), 2.29-2.32 (m, 6H), 2.41-2.91 (m, 5H), 3.51-3.76(m, 2H), 3.96-4.10 (m, 6H). LCMS: Rt: 1.327 min; MS m/z (ESI): 782.6 $[M+H]^+$.

Compound 02-11 was prepared in analogous fashion as Compound 02-10, using corresponding starting material.

Compound	Characterization
 Compound 02-11	^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.26-1.30 (m, 46H), 1.43-1.50 (m, 8H), 1.69-1.73 (m, 3H), 1.83-1.90 (m, 3H), 1.98-2.02 (m, 1H), 2.20-2.33 (m, 6H), 3.05-3.19 (m, 7H), 3.98-4.10 (m, 6H). LCMS: Rt: 1.205min; MS m/z (ESI): 781.7 $[M+H]^+$.

Example 6. Preparation of Compound 02-12

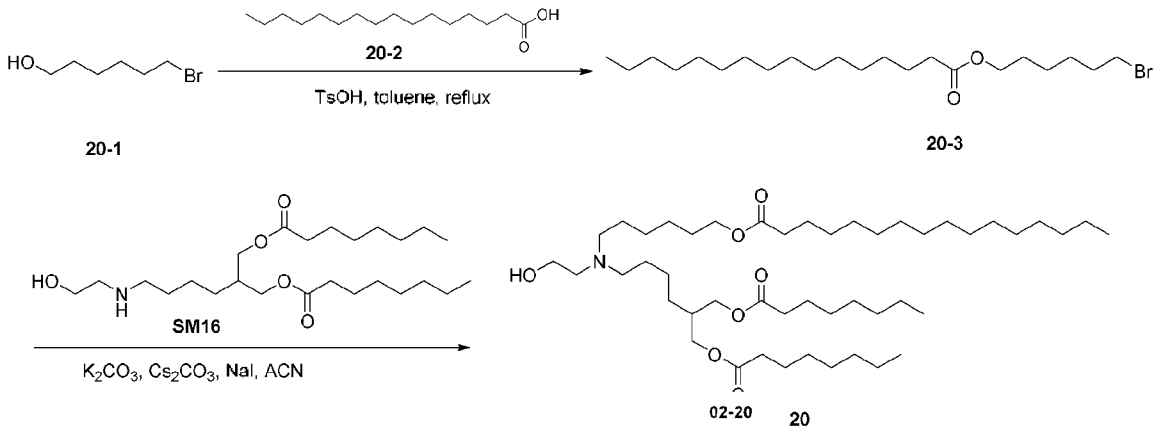
Compound 02-12 was prepared according to the scheme below.



Compound 02-12: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.89 (m, 18H), 1.25-1.35 (m, 53H), 1.41-1.48 (m, 8H), 1.56-1.61 (m, 20H), 1.95-2.01 (m, 2H), 2.28-2.35 (m, 6H), 2.43-2.46 (m, 4H), 2.56-2.58 (m, 2H), 3.51-3.54 (m, 2H), 4.00-4.10 (m, 8H). LCMS: Rt: 0.080 min; MS m/z (ESI): 1050.8 $[M+H]^+$.

Example 7. Preparation of Compound 02-20

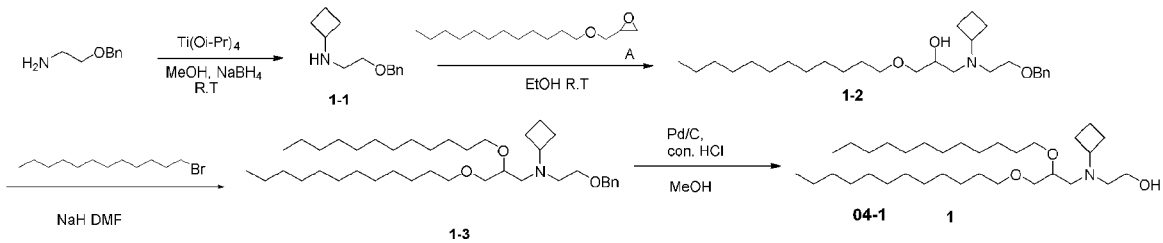
Compound 02-20 was prepared according to the scheme below.



Compound 02-20: ^{1}H NMR (400 MHz, CDCl3) δ : 0.86-0.90 (m, 9H), 1.25-1.36 (m, 48H), 1.41-1.48 (m, 5H), 1.60-1.62 (m, 8H), 1.97-2.00 (m, 1H), 2.27-2.32 (m, 6H), 2.43-2.46 (m, 4H), 2.56-2.59 (m, 2H), 3.52-3.54 (m, 2H), 4.01-4.10 (m, 6H). LCMS : Rt: 0.093 min; MS m/z (ESI): 782.6 $[M+H]^+$.

Example 8. Preparation of Compound 04-1

Compound 04-1 was prepared according to the scheme below.



LCMS for compound 1-1 of Example 8 - Rt: 0.750 min; MS m/z (ESI): 206.2 $[M+H]^+$.

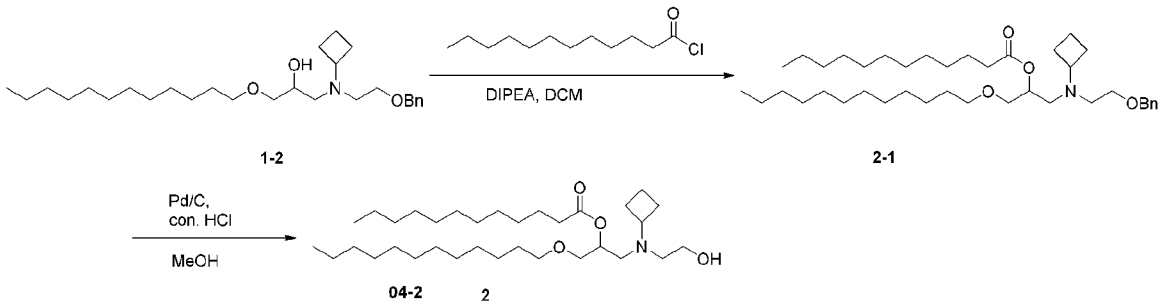
LCMS for compound 1-2 of Example 8 - Rt: 0.870 min; MS m/z (ESI): 448.3 $[M+H]^+$.

LCMS for compound 1-3 of Example 8 - Rt: 1.360 min; MS m/z (ESI): 616.5 $[M+H]^+$.

Compound 04-1: ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.79-0.83 (m, 6H), 1.14-1.26 (m, 38H), 1.47-1.61 (m, 6H), 1.86-1.96 (m, 4H), 2.51-2.58 (m, 4H), 3.17 (s, 1H), 3.32-3.44 (m, 5H), 3.51-3.66 (m, 3H). LCMS: Rt: 0.94 min; MS m/z (ESI): 526.5 $[M+H]^+$.

Example 9. Preparation of Compound 04-2

Compound 04-2 was prepared according to the scheme below.

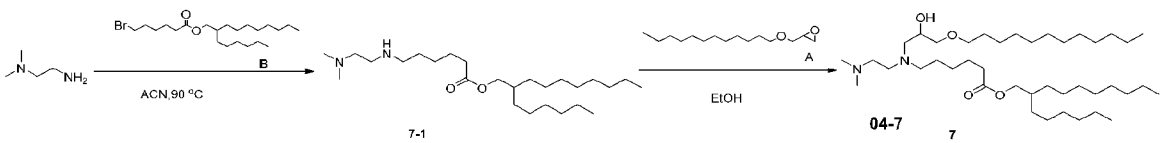


LCMS for compound 2-1 of Example 9 - Rt: 1.340 min; MS m/z (ESI): 630.5 $[M+H]^+$.

Compound 04-2: ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 6H), 1.25-1.33 (m, 35H), 1.50-1.69 (m, 7H), 1.87-1.99 (m, 1H), 2.00-2.08 (m, 2H), 2.33 (t, *J*=7.6 Hz, 2H), 2.56-2.81 (m, 4H), 3.17-3.27 (m, 1H), 3.38-3.48 (m, 3H), 3.50-3.65 (m, 3H), 5.08-5.14 (m, 1H). LCMS: Rt: 1.180 min; MS m/z (ESI): 540.4 $[M+H]^+$.

Example 10. Preparation of Compound 04-7

Compound 04-7 was prepared according to the scheme below.

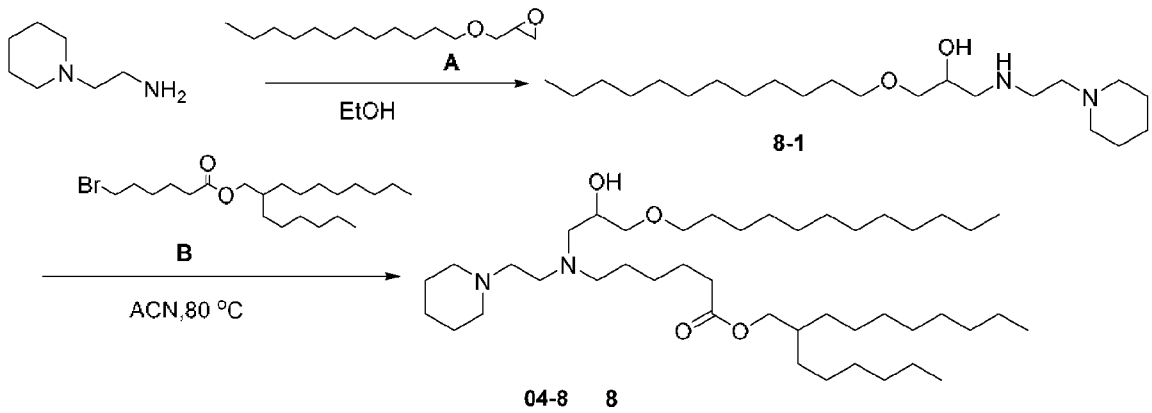


LCMS for compound 7-1 of Example 10 - LCMS: Rt: 0.780 min; MS m/z (ESI): 427.4 $[M+H]^+$.

Compound 04-7: ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 9H), 1.26-1.35 (m, 45H), 1.41-1.67 (m, 7H), 2.28-2.32 (m, 3H), 2.36-2.70 (m, 11H), 2.79-2.83 (m, 2H), 3.35-3.46 (m, 4H), 3.77-3.85 (m, 1H), 3.96-3.97 (m, 2H). LCMS: Rt: 1.220 min; MS m/z (ESI): 669.6 $[M+H]^+$.

Example 11. Preparation of Compound 04-8

Compound 04-8 was prepared according to the scheme below.

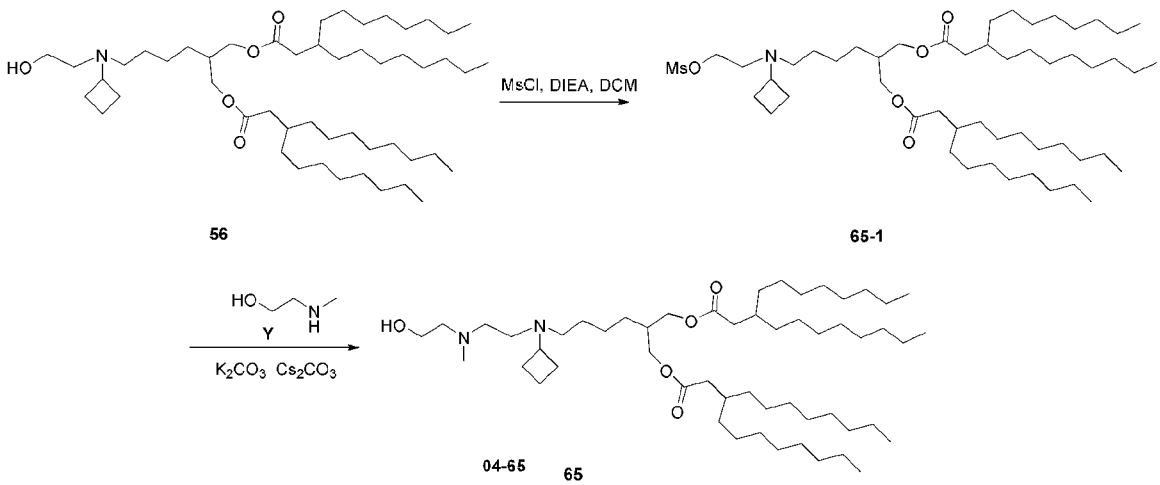


LCMS for compound 8-1 of Example 11 - LCMS: Rt: 0.730 min; MS m/z (ESI): 371.3 $[M+H]^+$.

Compound 04-8: ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 9H), 1.25-1.27 (m, 47H), 1.40-1.49 (m, 4H), 1.56-1.73 (m, 8H), 2.30 (t, *J*=7.6 Hz, 3H), 2.40-2.82 (m, 10H), 3.32-3.38 (m, 1H), 3.43-3.46 (m, 3H), 3.70-3.80 (m, 1H), 3.92-3.97 (m, 2H). LCMS: Rt: 1.090 min; MS m/z (ESI): 709.6 $[M+H]^+$.

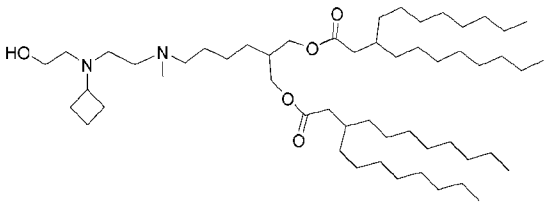
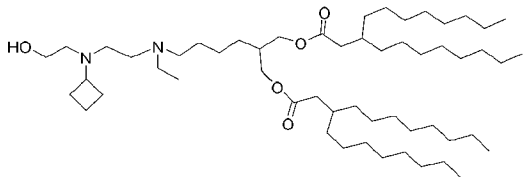
Example 12. Preparation of Compound 04-65, Compound 04-66 and Compound 04-67

Compound 04-65 was prepared according to the scheme below.



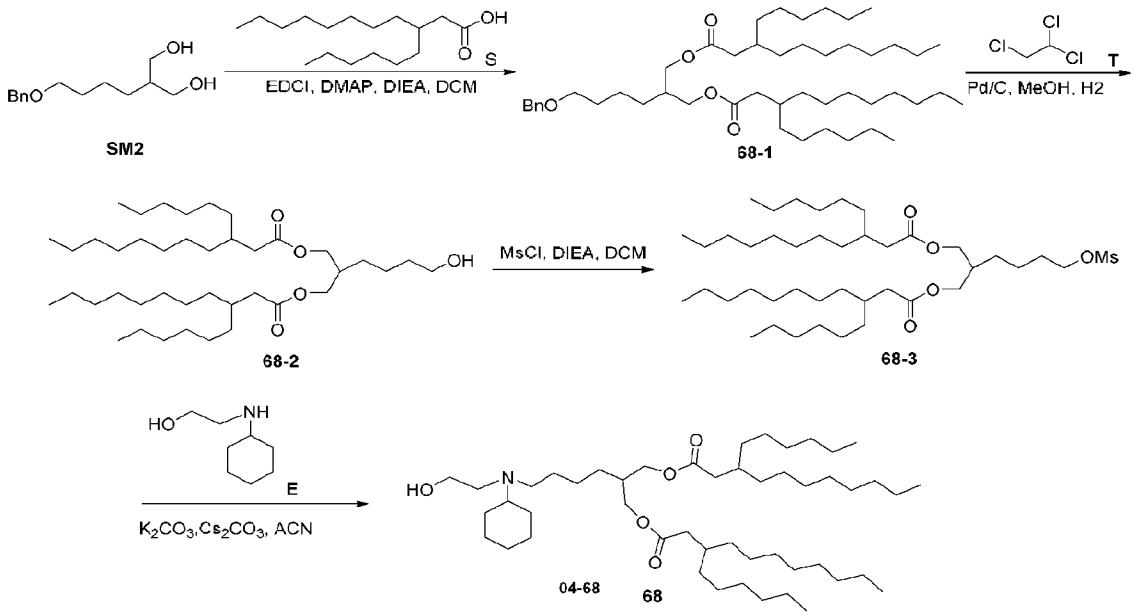
Compound 04-65: ^1H NMR (400 MHz, CCl_3D): δ: 0.79-0.83 (m, 12H), 1.23-1.27(m, 62H), 1.29-1.37 (m, 2H),1.51-1.61 (m, 2H), 1.76-1.93(m, 7H), 2.13-2.16 (m, 4H), 2.17-2.25 (m, 3H), 2.41-2.51 (m,7H), 3.05-3.06 (m, 1H), 3.52-3.54 (m. 2H), 3.92-4.03 (m, 4H). LCMS: Rt: 0.588 min; MS m/z (ESI):863.6 $[M+H]^+$.

Compounds 04-66 and 04-67 were prepared in analogous fashion as Compound 04-65, using corresponding starting material.

Compound	Characterization
 Compound 04-66	^{1}H NMR (400 MHz, CCl_3D): δ: 0.86-0.89 (m, 12H), 1.26-1.32(m, 58H), 1.33-1.51 (m, 8H), 1.82-2.02 (m, 7H), 2.22-2.25(m, 7H), 2.40-2.57 (m, 7H), 3.04-3.20 (m, 1H), 3.55-3.57 (m. 2H), 3.99-4.09 (m, 4H). LCMS: Rt: 0.588 min; MS m/z (ESI):863.6 $[M+H]^+$.
 Compound 04-67	^{1}H NMR (400 MHz, CCl_3D): δ: 0.86-0.89 (m, 12H), 1.25-1.29(m, 62H), 1.39-1.42 (m, 4H), 1.57-1.69(m, 3H), 1.77-1.85 (m, 2H), 1.96-2.10(m, 4H), 2.23-2.24(m, 4H), 2.50-3.18 (m, 11H), 3.61-3.75 (m, 2H), 4.02-4.07 (m, 4H). LCMS: Rt: 0.903 min; MS m/z (ESI):877.6 $[M+H]^+$.

Example 13. Preparation of Compound 04-68

Compound 04-68 was prepared according to the scheme below.

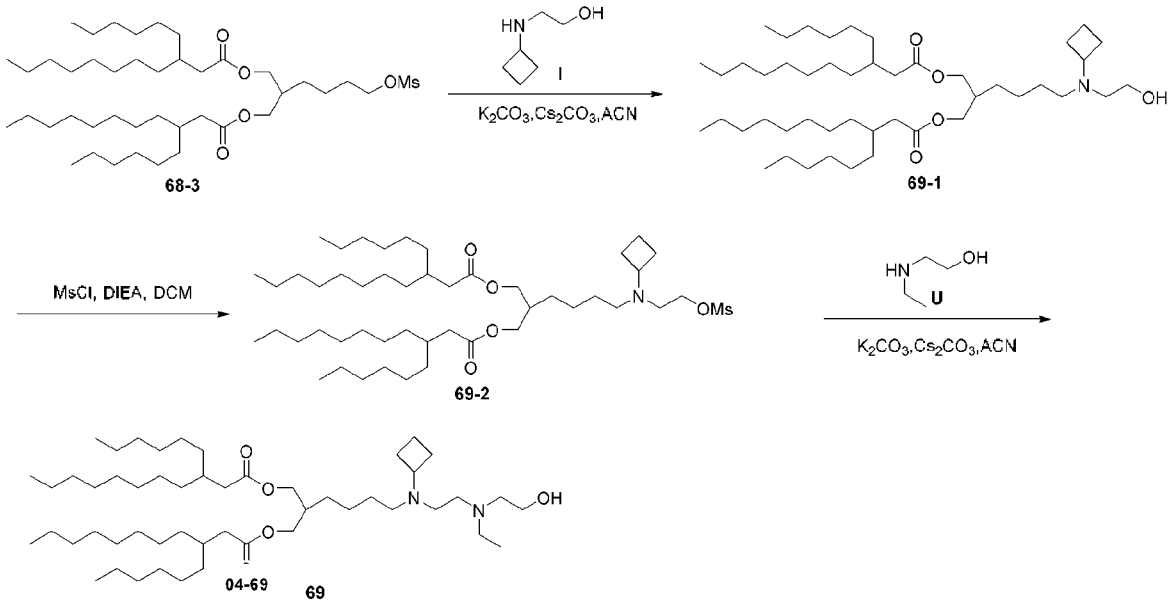


^{1}H NMR for compound 68-2 of Example 13 - ^{1}H NMR (400 MHz, CDCl3) δ: 0.86-0.90 (m, 12H), 1.26-1.46 (m, 53H), 1.56-1.62 (m, 2H), 1.83 (s, 2H), 1.96-2.02 (m, 1H), 2.23-2.24 (m, 4H), 3.64 (s, 2H), 4.02-4.11 (m, 4H).

Compound 04-68: ^{1}H NMR (400 MHz, CDCl3) δ: 0.83-0.92 (m, 12H), 1.17-1.37 (m, 56H), 1.38-1.45 (m, 2H), 1.64-1.67 (m, 2H), 1.70-1.86 (m, 6H), 1.92-2.04 (m, 2H), 2.19-2.26 (m, 4H), 2.40-2.49 (m, 3H), 2.57-2.65 (m, 2H), 3.41-3.51 (m, 2H), 3.97-4.12 (m, 4H). LCMS: Rt: 0.080 min; MS m/z (ESI): 778.5 $[M+H]^+$.

Example 14. Preparation of Compound 04-69, Compound 04-79 and Compound 04-80

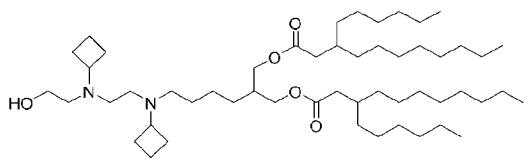
Compound 04-69 was prepared according to the scheme below.

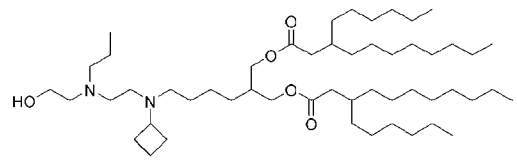


LCMS of compound 69-1 of Example 14 - Rt: 1.290 min; MS m/z (ESI): 750.7 $[M+H]^+$.

Compound 04-69: ^1H NMR (400 MHz, CDCl3) δ: 0.83-0.92 (m, 12H), 0.98-1.06 (m, 3H), 1.17-1.47 (m, 52H), 1.54-1.72 (m, 5H), 1.78-2.06 (m, 8H), 2.20-2.27 (m, 4H), 2.37-2.46 (m, 4H), 2.49-2.66 (m, 5H), 3.01-3.12 (m, 1H), 3.52-3.59 (m, 2H), 3.98-4.11 (m, 4H). LCMS: Rt: 0.093 min; MS m/z (ESI): 821.6 $[M+H]^+$.

Compounds 04-79 and 04-80 were prepared in an analogous fashion as compound 04-69, using corresponding starting material.

Compound	Characterization
 Compound 04-79	^1H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.90 (m, 12H), 1.26-1.47 (m, 55H), 1.58-1.70 (m, 4H), 1.82-2.01 (m, 11H), 2.22-2.24 (m, 4H), 2.43-2.58 (m, 8H), 3.13-3.26 (m, 2H), 3.58 (s, 2H), 3.99-4.11 (m, 4H). LCMS: Rt:1.870 min; MS m/z (ESI): 847.7 $[M+H]^+$.

 Compound 04-80	^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86-0.91 (m, 15H), 1.26-1.48 (m, 60H), 1.82-2.02 (m, 8H), 2.22-2.24 (m, 4H), 2.49-2.65 (m, 8H), 3.10-3.15 (m, 1H), 3.60 (s, 2H), 3.99-4.11 (m, 4H). LCMS: Rt:1.850 min; MS m/z (ESI): 835.7 $[M+H]^+$.

Example 15. Initial screening of LNPs comprising a steroid containing phospholipid

Briefly, the specified amounts of the lipid components were solubilized in ethanol at the specified molar ratios (see Table 6). The mRNA was diluted in 10 to 50 mM citrate buffer, pH = 3-5. The LNPs were prepared at a total lipid to mRNA weight ratio of approximately 10:1 to 30:1 by mixing the ethanolic lipid solution with the aqueous mRNA solution at a volume ratio of 1:3 using a microfluidic apparatus, total flow rate ranging from 9-30 mL/min. Ethanol was thereby removed and replaced by Dulbecco's phosphate-buffered saline (DPBS) using dialysis. Finally, the lipid nanoparticles were filtered through a 0.2 μm sterile filter.

Lipid nanoparticle size were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern UK) using a 173° backscatter detection mode. The encapsulation efficiency of lipid nanoparticles was determined using a Quant-it Ribogreen RNA quantification assay kit (Thermo Fisher Scientific, UK) according to the manufacturer's instructions.

To measure the size and polydispersibility index (PDI) of lipid nanoparticle formulations were diluted 20-fold in PBS and transferred 1 mL in measurement cuvette. The LNP encapsulation efficiency (EE%) was determined using a Quant-it RiboGreen RNA assay kit, LNP formulations were diluted to 0.5 μg/mL in Tris-EDTA and 0.1% Triton respectively. In order to determine free RNA and total RNA fluorescence intensity, ribogreen reagent were diluted 200-fold with Tris-EDTA buffer and mix at the same volume as diluted LNP formulation. Fluorescence intensity was measured at room temperature in a Molecular Devices Spectramax iD3 spectrometer using excitation and emission wavelengths of 488 nm and 525 nm. EE% was calculated based on the ratio of encapsulated to total RNA fluorescence intensity.

Table 6: Expression levels of LNPs comprising a steroid containing phospholipid

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)	EPO expression Level (μg/mL)
compound 01-1/DSPC/Chol/DMG-PEG	45/10/43.5/1.5	68	0.051	89.2	10.42
compound 01-1/PChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	87.57	0.031	92.4	34.81
compound 01-1/OChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	91.57	0.016	94.5	27.94

Lipid nanoparticles encapsulating human erythropoietin (hEPO) mRNA were prepared as described above, and systemically administered to 6-8 week old female ICR mice (Xipuer-Bikai, Shanghai) at 0.5mg/kg dose by tail vein injection. Mice were euthanized by CO_2 overdoses at 6 hours post administration, and blood samples were taken for hEPO measurement. Particularly, serum was separated from total blood by centrifugation at 5000g for 10 minutes at 4 °C, snap-frozen and stored at -80 °C for analysis. The serum hEPO level was measured using an ELISA assay carried out using a commercial kit (DEP00, R&D systems) according to manufacturer's instructions. The hEPO expression levels (μg/ml) measured from the tested group are plotted in FIG. 1 and summarized in Table 6.

As shown, replacing DSPC with PChemsPC and adjustment of the molar ratio significantly increased EPO-LNP *in-vivo* protein expression by 2-3 fold.

Example 16. Expression levels of loaded LNPs comprising a steroid containing phospholipid

This study examined *in vivo* expression levels of LNP formulations containing 45% - 75% compound 01-1 with DSPC or PChemsPC. Lipid nanoparticles containing human erythropoietin (hEPO) mRNA were prepared as described in Example 15. Results are shown in FIG. 2 and Table 7.

Of the DSPC LNPs tested, LNPs containing 45-60 mol% compound 01-1 resulted in the highest protein expression level. For PChemsPC LNPs, 60 -75 mol% compound 01-1 resulted in the highest protein expression level.

Table 7. Further expression levels of LNPs comprising a steroid containing phospholipid

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)	EPO expression Level (μg/mL)
compound 01-1/DSPC/Chol/DMG-PEG	45/10/43.5/1.5	65.3	0.072	88.73	4.83
compound 01-1/DSPC/Chol/DMG-PEG	50/10/38.5/1.5	68.18	0.08	91.40	8.07
compound 01-1/DSPC/Chol/DMG-PEG	60/10/28.5/1.5	75.06	0.046	90.40	8.67
compound 01-1/DSPC/Chol/DMG-PEG	65/10/23.5/1.5	86.15	0.016	90.35	3.23
compound 01-1/DSPC/Chol/DMG-PEG	70/10/18.5/1.5	94.4	0.002	85.61	1.55
compound 01-1/PChemsPC/Chol/DMG-PEG	50/10/38.5/1.5	64.92	0.041	92.93	5.72
compound 01-1/PChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	83.19	0.034	87.68	16.91
compound 01-1/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	88.12	0.04	85.74	18.13
compound 01-1/PChemsPC/Chol/DMG-PEG	70/5/23.5/1.5	117.2	0.004	90.49	13.95
compound 01-1/PChemsPC/Chol/DMG-PEG	75/5/18.5/1.5	141.3	0.008	85.5	9.11

Example 17. Screen of %PChemsPC for optimal protein expression

A further screen was conducted to examine *in vivo* expression levels of LNP formulations containing 5- 25 mol% PChemsPC with different amounts of compound 01-1. Lipid nanoparticles containing human erythropoietin (hEPO) mRNA were prepared as described in Example 15. Results are shown in FIG. 3 and Table 8.

Of the PChemsPC LNPs tested, 5-10 mol% PChemsPC had the highest protein expression level. The optimal amount of PChemsPC varied with the amount of compound 01-1. When the mol% of PChemsPC exceeded 15%, protein expression appeared to decrease.

Table 8. Additional expression levels of LNPs comprising a steroid containing phospholipid.

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)	EPO expression Level (μg/mL)
compound 01-1/DSPC/Chol/DMG-PEG	50/10/38.5/1.5	68.03	0.028	90.77	8.07
compound 01-1/PChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	83.19	0.034	87.68	16.91
compound 01-1/PChemsPC/Chol/DMG-PEG	65/5/28.5/1.5	95.03	0.014	86.90	12.42
compound 01-1/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	88.12	0.04	85.74	18.13
compound 01-1/PChemsPC/Chol/DMG-PEG	65/15/18.5/1.5	77.42	0.033	84.32	5.60
compound 01-1/PChemsPC/Chol/DMG-PEG	65/20/13.5/1.5	88.13	0.02	85.94	2.63
compound 01-1/PChemsPC/Chol/DMG-PEG	65/25/8.5/1.5	84.9	0.014	91.10	2.42
compound 01-1/PChemsPC/Chol/DMG-PEG	70/5/23.5/1.5	117.2	0.004	90.49	13.95
compound 01-1/PChemsPC/Chol/DMG-PEG	70/10/18.5/1.5	89.08	0.049	90.09	9.63
compound 01-1/PChemsPC/Chol/DMG-PEG	70/15/13.5/1.5	83.74	0.042	86.40	3.07
compound 01-1/PChemsPC/Chol/DMG-PEG	70/20/8.5/1.5	90.38	0.002	86.82	1.82

Example 18. Screen of LNP compositions with a steroid containing phospholipid for optimal protein expression

This study examined *in-vivo* EPO expression level of PChemsPC LNPs with different compositions. Lipid nanoparticles containing human erythropoietin (hEPO) mRNA were prepared as described in Example 15. In this study, a molar ratio of 50% -75% compound 01-1, 18.5% - 38.5% cholesterol, 5-15% PChemsPC were tested to achieve acceptable protein expression levels. Corresponding LNP characterizations were listed in Table 9.

Table 9. Additional expression levels of LNPs comprising a steroid containing phospholipid with different molar ratios.

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)	EPO expression Level (μg/mL)
Compound 01-1 /DSPC/Chol/DMG-PEG	50/10/38.5/1.5	68.03	0.028	90.77	8.07
Compound 01-1 /PChemsPC/Chol/DMG-PEG	50/10/38.5/1.5	64.92	0.041	92.93	5.72
Compound 01-1 /PChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	83.19	0.034	87.68	16.91
Compound 01-1 /PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	88.12	0.04	85.74	18.13
Compound 01-1 /PChemsPC/Chol/DMG-PEG	65/15/18.5/1.5	77.42	0.033	84.32	5.6
Compound 01-1 /PChemsPC/Chol/DMG-PEG	70/10/18.5/1.5	89.08	0.049	90.09	9.63
Compound 01-1 /PChemsPC/Chol/DMG-PEG	70/5/23.5/1.5	117.2	0.004	90.49	13.95
Compound 01-1 /PChemsPC/Chol/DMG-PEG	75/5/18.5/1.5	116.8	0.02	82.63	9.11

It can be seen from Table 9 that within LNP formulations comprsing 60-75 mol% cationic lipids, 5-10 mol% PChemsPC, and 18.5-28.5 mol% cholesterol can result in a high protein expression and the physicochemical properties thereof such as sizes, polydispersibility index and encapsulation efficiency are acceptable.

Example 19. Tissue-specific Expression of Nucleic Acid Molecules Delivered in LNP formulations.

To study the tissue biodistribution of LNPs in mice, LNP formulations listed in Table 10 containing mRNA encoding luciferase were prepared as described in Example 15.

Table 10. LNP compositions and physical characterizations

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)
Compound 01-1 /DSPC/Chol/DMG-PEG	50/10/38.5/1.5	64.18	0.05	93.82
Compound 01-1 /PChemsPC/Chol/DMG-PEG	50/10/38.5/1.5	70.87	0.049	94.88
Compound 01-1 /PChemsPC/Chol/DMG-PEG	60/10/28.5/1.5	82.29	0.018	93.35

Compound 01-1 /PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	86.56	0.005	92.57
Compound 01-1 /PChemsPC/Chol/DMG-PEG	70/5/23.5/1.5	96.68	0.009	90.49
Compound 01-1 /PChemsPC/Chol/DMG-PEG	75/5/18.5/1.5	108.7	0.017	91.20
Compound 01-1 /DChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	84.98	0.004	94.55
Compound 01-1 /OChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	85.32	0.031	92.20

Each formulation was systematically administered to 6-8 week old female ICR mice (Xipuer-Bikai, Shanghai) at a 0.25 mg/kg dose by tail vein injection. After 6 hours, the mice were subcutaneously administered with XenoLight D-luciferin (potassium salt), a substrate of luciferase that catalyze the production of luminescence. The mice were subsequently euthanized by CO_2 overdoses 15 min thereafter. Mice tissues were harvested and placed in a luminescence imaging scanner to measure the expression level of luciferase in each tissue. The luminescence levels measured from harvest liver tissues were plotted in FIG.4, showing the mean value and standard deviation (SD) of at least five repeated tested animals for each group.

As shown in FIG.4, LNPs composed of a steroid containing phospholipid (e.g. PChemsPC, OChemsPC, DChemsPC) yield higher liver signal, in comparison to DSPC control. A molar ratio of 50% -75% compound 01-1 with PChemsPC yields higher liver luminescence signal.

The percentage of luminescence intensity in different tissues were calculated and plotted in FIG. 5.

As shown in FIG. 5, after injection, DSPC LNP leads to 96% liver distribution with around 3% spleen distribution, while steroid containing phospholipid LNP shows 98 -99% liver distribution, with 0.4% spleen distribution. It can be seen that steroid-modified phospholipid LNP reveals better liver-tropism.

Example 20. Characterization of sterol-modified phospholipid LNP with different ionizable lipids

LNP formulations containing PChemsPC were prepared with different ionizable lipids. The LNP formulations were composed of a ionizable lipid at a molar ratio of 65%, a PChemsPC lipid at a molar ratio of 10%, a cholesterol-based lipid at a molar ratio of 23.5% and a PEGylated lipid at a molar ratio of 1.5%. Lipid nanoparticles containing human erythropoietin (hEPO) mRNA were prepared as described in Example 15.

Compound 01-1with DSPC control was used as a comparison group here in this study. Corresponding LNP characterizations with different ionizable lipids were listed in Table 11.

Table 11. LNP characterizations with different ionizable lipids.

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)	EPO expression Level (μg/mL)
Compound 01-1 /DSPC/Chol/DMG-PEG	50/10/38.5/1.5	68.18	0.08	91.40	8.07
Compound 01-1 /PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	88.12	0.04	85.74	18.13
Lipid 5/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	91.05	0.095	93.40	12.23
SM-102/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	87.01	0.079	95.70	11.85
ALC-0315/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	86.62	0.091	87.46	27.99
Compound 02-3/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	99.91	0.064	78.77	31.18
Compound 02-6/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	108.6	0.127	81.44	30.76
Compound 02-22/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	85.83	0.078	89.22	10.80
Compound 02-65/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	98.12	0.103	82.44	27.53
Compound 03-190/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	106.1	0.146	87.90	21.72
Compound 03-135/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	99.26	0.062	89.70	17.22
Compound 03-140/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	107.5	0.085	77.50	20.91
Compound 03-143/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	112	0.069	76.10	26.54
Compound 03-145/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	125.4	0.090	61.70	14.97

As shown in Table 11, most of the ionizable lipids show better protein expression level compared to compound 01-1 control, indicates that PChemsPC is widely compatible with most

of the ionizable lipids. The combination of PChemsPC and Compound 02-3 can yield at most 2.8-fold protein expression level escalation.

Additionally, for commercial lipids ALC-0315 and MC3, the effect of replacement of DSPC with PChemsPC on in-vivo protein expression level was also studied. In this study, the LNP formulations were composed of an ionizable lipid at a molar ratio of 50% - 65%, a phospholipid at a molar ratio of 10%, a cholesterol-based lipid at a molar ratio of 23.5% - 38.5% and a PEGylated lipid at a molar ratio of 1.5%. LNP characterizations were listed in Table 12.

Table 12. Physical characterizations of ALC-0315 and MC3 LNPs.

Lipid Components	Molar Ratio	Size (nm)	PDI	EE(%)
ALC-0315/DSPC/Chol/DMG-PEG	50/10/38.5/1.5	67.53	0.123	95.40
ALC-0315/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	86.62	0.091	87.46
MC3/DSPC/Chol/DMG-PEG	50/10/38.5/1.5	72.76	0.102	98.80
MC3/PChemsPC/Chol/DMG-PEG	65/10/23.5/1.5	92.83	0.011	99.20

In vivo hEPO expression levels fold change were depicted in FIG. 6.

As shown in FIG.6, replacement of DSPC with PChemsPC can significantly improve in-vivo protein expression level by 1.6-1.8 fold.

Example 21. Characterization of in vivo serum cytokines post injection of a steroid containing phospholipid LNP

As an allogenic substance, LNP injection will lead to significant increase of pro-inflammatory cytokines, such as inteleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interferon-alpha (IFN-α), which can cause innate immune response and leads to undesirable side effects. This study evaluates serum cytokine levels after LNP systematically administration, comparing the serum cytokine boosted by DSPC LNP and PChemsPC LNP.

Lipid nanoparticles containing human erythropoietin (hEPO) mRNA were prepared as described in Example 15, and systemically administered to 6-8 week old female ICR mice (Xipuer-Bikai, Shanghai) at 0.5mg/kg dose by tail vein injection. Mice were euthanized by CO_2 overdoses at 6 hours post administration, and blood samples were taken for cytokines measurement.

Particularly, sera were separated from total blood by centrifugation at 5000g for 10 minutes at 4 °C, snap-frozen and stored at -80 °C for analysis.

DSPC LNPs were composed of ionizable lipid with a molar ratio of 50%, DSPC with a molar ratio of 10%, cholesterol with a molar ratio of 38.5% and a molar ratio of 1.5% for PEGylated lipid. PChemsPC LNPs were composed of ionizable lipid with a molar ratio of 65%, PChemsPC with a molar ratio of 10%, a molar ratio of 23.5% and 1.5% for cholesterol and PEGylated lipids respectively. Several ionizable lipids (e.g. compound 01-1, lipid 5, SM-102, ALC-0315, Compound 03-135) were tested in this study.

The results were shown in FIGs. 7-11. As shown in FIGs. 7-11, PChemsPC LNP can singnificantly deminish IL-6 levels compared to corresponding DSPC control. Especially for ALC-0315 LNP, around 9-fold drop of IL-6 level were observed, indicating that PChemsPC LNPs have better safety post administration.

Example 22. Characterization of in vivo serum cytokines after administration of a steroid containing phospholipid LNP with self-amplifying mRNA (saRNA)

In this study, lipid nanoparticles containing human erythropoietin (hEPO) self-amplifying mRNA were prepared as described in Example 15. After tail vein injection, mice were euthanized by CO_2 overdoses at 6 hours post administration, and blood samples were taken for cytokines measurement. Cytokine levels were measured and plotted in FIG. 12 and FIG. 13.

As shown in FIG. 12 and FIG. 13, PChemsPC LNPs lead to significantly lower IL-6, IFN-α, TNF-α levels.

Example 23. Characterization of sterol-modified phospholipid LNP with CD3-CD19 mRNA

In this study, DSPC LNPs were composed of compound 01-1 with a molar ratio of 50%, DSPC with a molar ratio of 10%, cholesterol with a molar ratio of 38.5% and a molar ratio of 1.5% for PEGylated lipid. PChemsPC LNPs were composed of compound 01-1 with a molar ratio of 65%, PChemsPC with a molar ratio of 10%, a molar ratio of 23.5% and 1.5% for cholesterol and PEGylated lipids respectively.

Lipid nanoparticles encapsulating CD19-CD3 mRNA were prepared as described in Example 15, and systemically administered to 6-8 week old female Balb/c mice (Xipuer-Bikai, Shanghai) at 0.3mg/kg dose by tail vein injection. Mice were euthanized by CO_2 overdoses at 6

hours post administration, and blood samples were taken for antibody measurement. Particularly, serum was separated from total blood by centrifugation at 5000g for 10 minutes at 4 °C, snap-frozen and stored at -80 °C for analysis. The serum antibody level was shown in FIG. 14.

As shown in FIG.14, PChemsPC LNP can significantly improve CD3-CD19 antibody expression level post administration.

CLAIMS

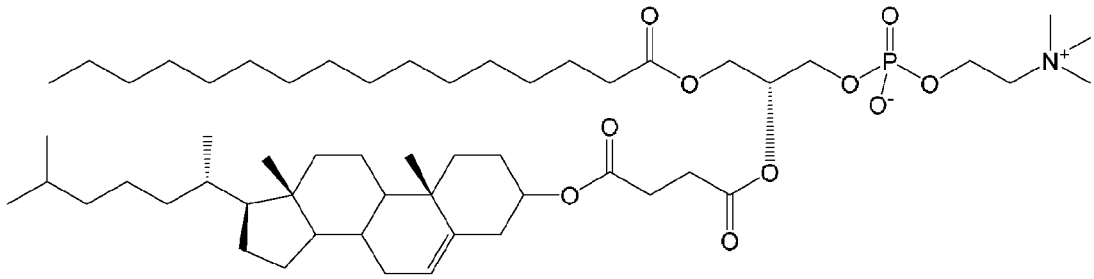
1. A lipid nanoparticle (LNP) comprising

a phospholipid containing a sterol moiety;

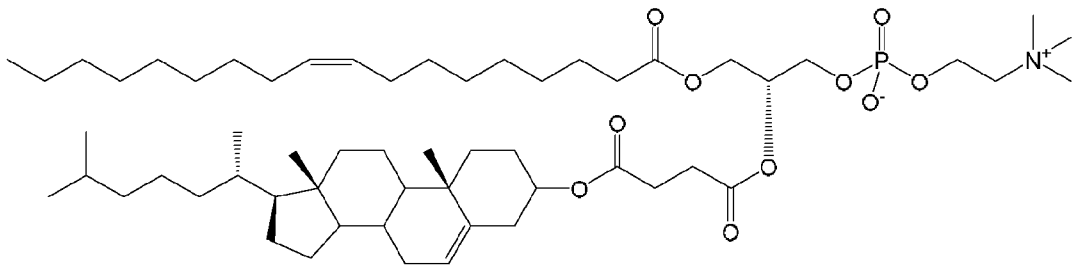
an ionizable lipid; and

a polymer conjugated lipid.

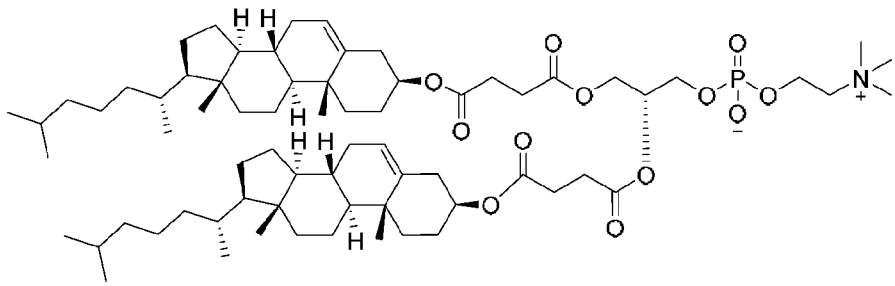
2. The LNP of claim 1, wherein the phospholipid has a structure selected from:



,



, and



.

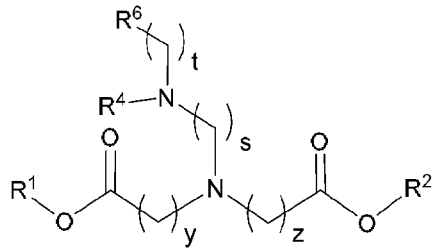
3. The LNP of claim 1 or 2, wherein the phospholipid comprises from 1 to 30 mol%, preferably from 2 to 25 mol%, preferably from 3 to 20 mol%, more preferably from 5 to 15 mol% of the total amount of lipids in the LNP.

4. The LNP of any one of claims 1 to 3, wherein the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1, preferably from 18:1 to 2.5:1, preferably from 16:1 to 4:1, more preferably from 15:1 to 5:1.

5. The LNP of any one of claims 1 to 4, wherein the ionizable lipid is a cationic lipid, preferably the ionizable lipid is a compound according to any one of the formula selected from

01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, more preferably the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5.

6. The LNP of any one of claims 1 to 4, wherein the ionizable lipid is selected from compounds of Formula (01-I-O):



(01-I-O),

wherein y and z are each independently an integer from 4 to 6,

s is an integer from 2 to 4,

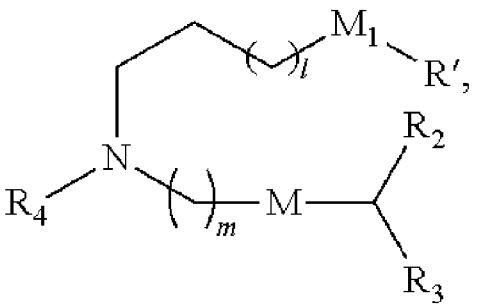
t is an integer from 1 to 3, and

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^4 is C_3-C_8 cycloalkyl;

R^6 is hydrogen or hydroxyl,

compounds of Formula 05-I:



wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

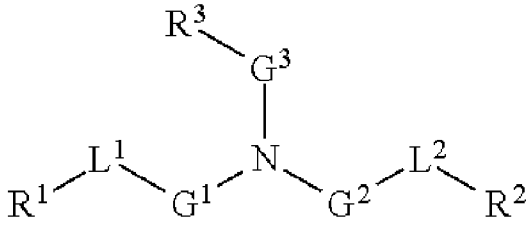
M_1 is -C(O)O-;

R_4 is $-(CH_2)_nOH$, and n is selected from 1, 2, 3, 4, or 5;

M is -OC(O)-; and

R_2 and R_3 are both C_{6-10} alkyl,

compounds of Formula (06-I):



(06-I)

wherein

L^1 and L^2 is -O(C=O)-;

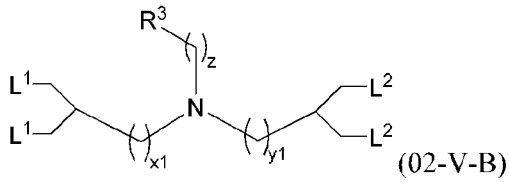
G^1 and G^2 are each independently unsubstituted C_4-C_8 alkylene;

G^3 is C_3-C_8 alkylene;

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^3 is H or OH,

Compounds of Formula (02-V-B)



(02-V-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alky;

R^3 is -OR^6;

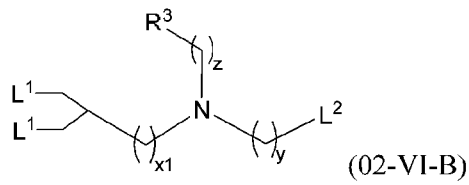
R^6 is hydrogen;

z is an integer from 2 to 12;

x1 is an integer from 0 to 9;

y1 is an integer from 0 to 9;

Compounds of Formula (02-VI-F)



(02-VI-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^3 is -OR^6;

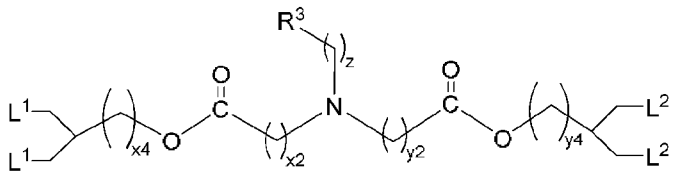
R^6 is hydrogen;

z is an integer from 2 to 12;

y is an integer from 2 to 12;

x1 is an integer from 2 to 5;

Compounds of Formula (02-V-F)



(02-V-F)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl;

R^3 is -OR^6;

R^6 is hydrogen;

z is an integer from 2 to 12;

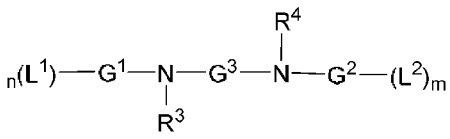
x2 is an integer from 2 to 9;

x4 is an integer from 0 to 3;

y2 is an integer from 2 to 9;

y4 is an integer from 0 to 3,

Compounds of formula (03-I)



(03-I),

wherein

G^1 and G^2 are each independently C_3-C_8 alkylene;

each L^1 is independently –OC(=O)R^1 or -C(=O)OR^1;

each L^2 is independently -C(=O)OR^2 or –OC(=O)R^2;

R^1 is independently C_6-C_{24} alkyl;

R^2 is independently C_6-C_{24} alkyl;

G^3 is C_2-C_{12} alkylene;

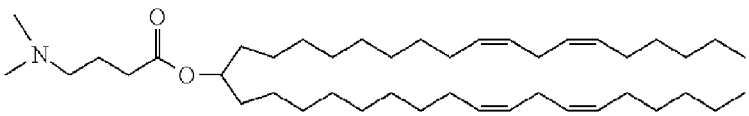
R^3 is C_3-C_8 cycloalkyl;

R^4 is C_1-C_4 hydroxylalkyl;

n is 1 or 2;

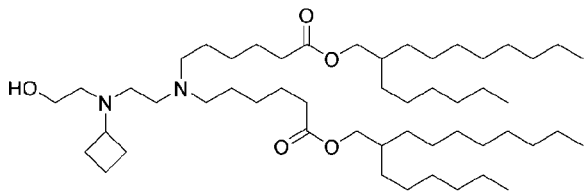
m is 1 or 2, and

compound 07-I:

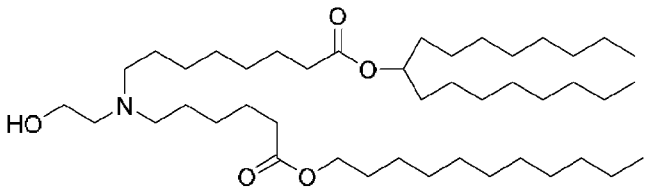


(compound 07-I).

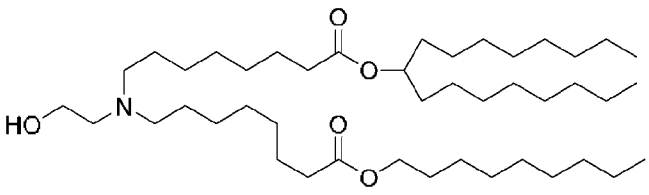
7. The LNP of claim 6, wherein the ionizable lipid is selected from the following compounds:



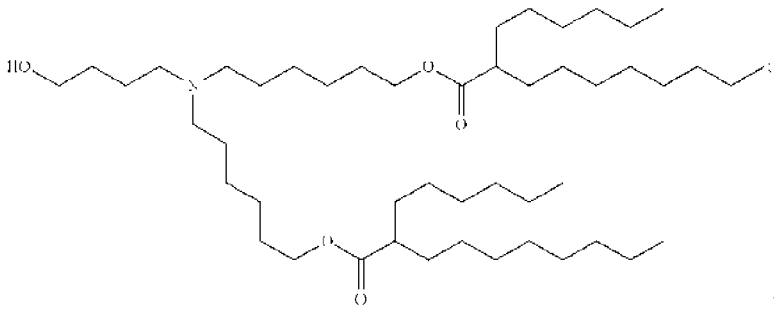
(Compound 01-1)



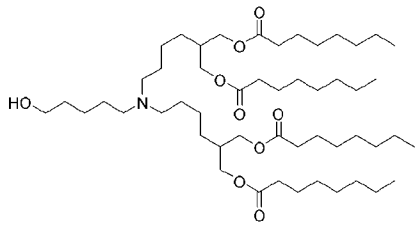
(SM102)



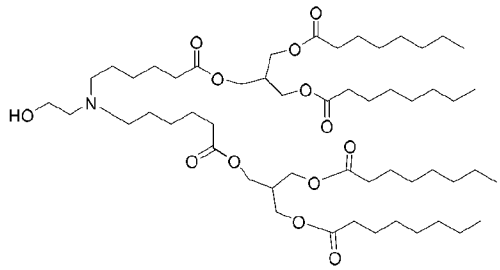
(Lipid 5)



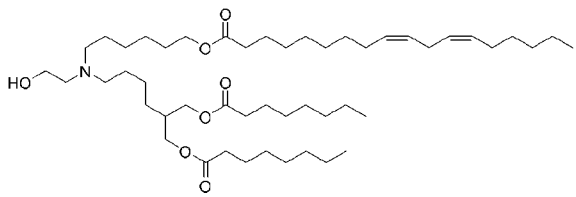
(Compound 06-1)



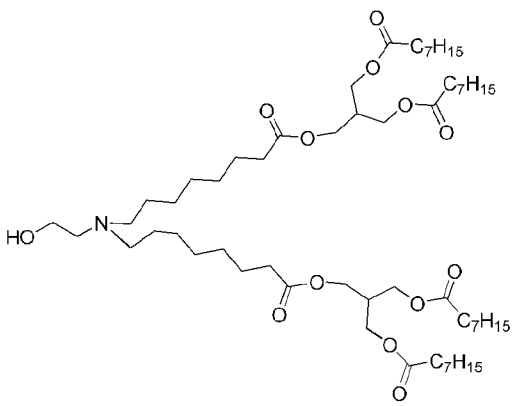
(Compound 02-3)



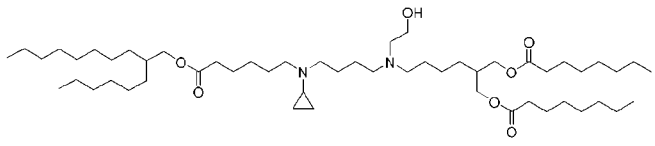
(Compound 02-6)



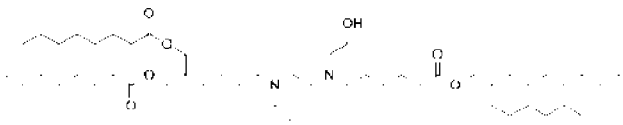
(Compound 02-22)



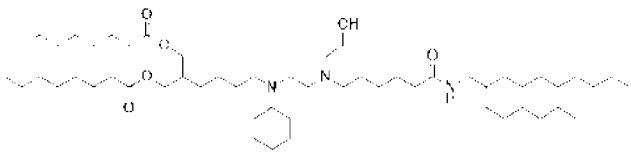
(Compound 02-65)



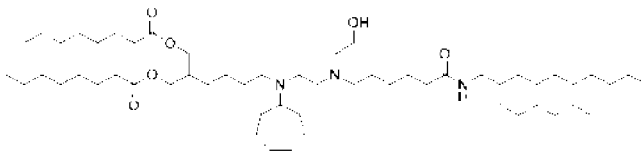
(Compound 03-190)



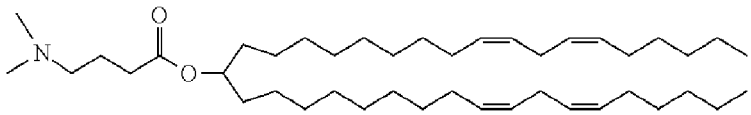
(Compound 03-135)



(Compound 03-140)



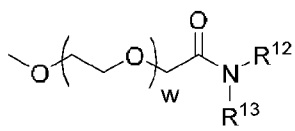
(Compound 03-143), and



(Compound 07-I).

8. The LNP of any one of claims 1 to 7, wherein the ionizable lipid comprises from 40 to 80 mol%, from 45 to 75 mol%, from 50 to 70 mol%, or from 60 to 65 mol% of a total amount of lipids in the LNP.

9. The LNP of any one of claims 1 to 8, wherein the polymer conjugated lipid is a PEGylated lipid, preferably with the structure:



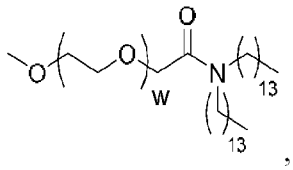
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60, preferably from 45 to 55, more preferably w is 49.

10. The LNP of any one of claims 1 to 9, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60, preferably from 45 to 55, more preferably w is 49, preferably the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

11. The LNP of any one of claims 1 to 10, wherein the polymer conjugated lipid comprises from 0.5 to 5 mol%, preferably from 1 to 2 mol%, more preferably 1.5 mol% of the total amount of lipids in the LNP.

12. The LNP of any one of claims 1 to 11, wherein the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid of from 1:2 to 1:20, preferably from 1:3 to 1:18 or from 1:5 to 1:10.

13. The LNP of any one of claims 1 to 12, further comprising a lipid stabilizer.

14. The LNP of claim 13, wherein the lipid stabilizer is selected from sterols, corticosteroids, tomatidine, tomatine, ursolic acid, and alpha-tocopherol, preferably selected from cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, prednisolone, dexamethasone, prednisone, hydrocortisone, tomatidine, tomatine, ursolic acid, and alpha-tocopherol.

15. The LNP of claim 13 or 14, wherein the lipid stabilizer comprises from 5 to 50 mol%, preferably from 8 to 40 mol%, more preferably from 10 to 30 mol% of a total amount of lipids in the LNP.

16. The LNP of any of claims 13 to 15, wherein the LNP has a molar ratio of the lipid stabilizer to the phospholipid of from 10:1 to 1:4, preferably from 5:1 to 1:3.

17. The LNP of any one of claims 1 to 16, wherein the LNP has a size of from 20 nm to 300 nm, preferably from 50 nm to 150 nm, preferably from 60 nm to 140 nm, more preferably from 80 nm to 100 nm, even more preferably from 85 nm to 95 nm, as determined using dynamic light scattering.

18. A composition comprising lipid nanoparticles (LNPs) and a therapeutic payload, wherein each LNP is an LNP of any one of claims 1 to 17.

19. The composition of claim 18, wherein at least 80%, preferably at least 85% of the LNPs encapsulate the therapeutic payload.

20. The composition of claim 18 or 19, wherein the therapeutic payload is selected from nucleic acids, preferably selected from DNAs and RNAs, more preferably selected from catalytic DNA, plasmid DNA, aptamer, complementary DNA, mRNA, antisense oligonucleotide, miRNA, miRNA inhibitor, micRNA, multivalent RNA, dsRNA, shRNA, antisense RNA, tRNA, aiRNA, a ribozyme, an aptamer, and a vector.

21. The composition of claim 18 or 19, wherein the therapeutic payload is a mRNA.

22. A method for expressing protein in a cell, comprising introducing the composition of claim 21 to the cell.

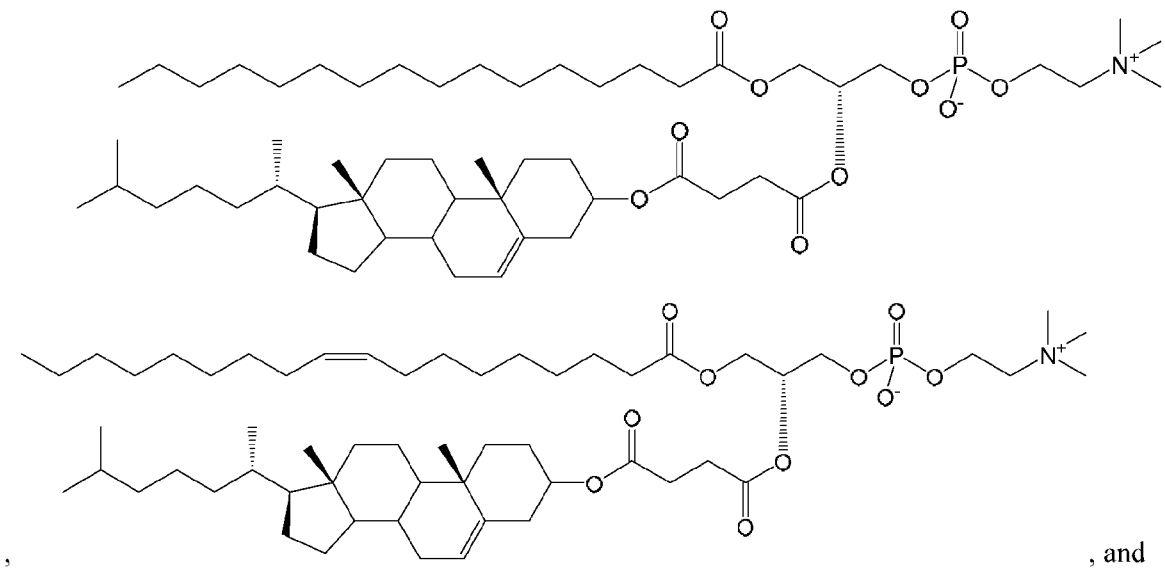
23. The method of claim 22, wherein the cell is a mammalian cell.

24. A method for delivering a protein to a subject, comprising administering the composition of claim 21 to the subject, wherein the mRNA encodes the protein.

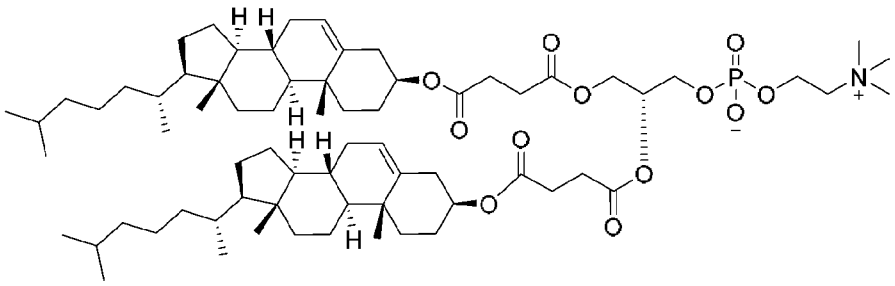
25. The method of claim 24, wherein the composition is administered systemically.

26. The method of claim 24 or 25, wherein the subject is a mammal, in particular, a human.

27. A lipid nanoparticle (LNP) comprising a phospholipid, wherein the phospholipid has a structure selected from:



, and



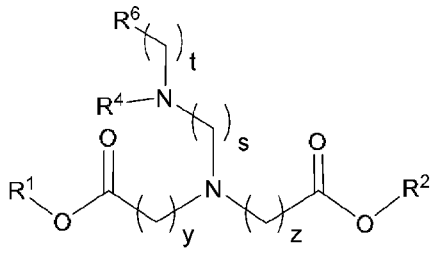
28. The LNP of claim 27, further comprising an ionizable lipid.

29. The LNP of claim 28, wherein the LNP has a molar ratio of the ionizable lipid to the phospholipid from 20:1 to 2:1, preferably from 18:1 to 2.5:1, preferably from 16:1 to 4:1, more preferably from 15:1 to 5:1.

30. The LNP of claim 27 or 28, wherein the ionizable lipid comprises from 40 to 80 mol%, from 45 to 75 mol%, from 50 to 70 mol%, or from 60 to 65 mol% of a total amount of lipids in the LNP.

31. The LNP of any one of claims 28 to 30, wherein the ionizable lipid is a cationic lipid, preferably the ionizable lipid is a compound according to any one of the formula selected from 01-I, 01-II, 02-I, 02-II, 03-I, 03-II-A, 03-II-B, 03-II-C, 03-II-D, 04-I, 04-III, 04-IV, 05-I, 06-I, and sub-formulas thereof, more preferably the ionizable lipid is a cationic lipid selected from the compounds listed in any one of Tables 1 to 5.

32. The LNP of any one of claims 28 to 30, wherein the ionizable lipid is selected from compounds of Formula (01-I-O):



(01-I-O),

wherein y and z are each independently an integer from 4 to 6,

s is an integer from 2 to 4,

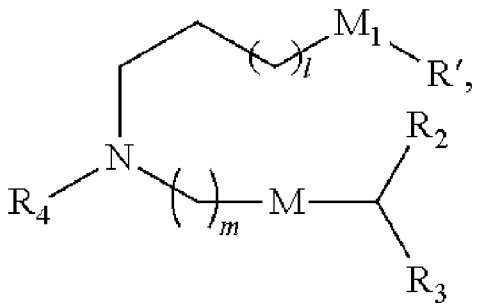
t is an integer from 1 to 3, and

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^4 is C_3-C_8 cycloalkyl;

R^6 is hydrogen or hydroxyl,

compounds of Formula 05-I:



wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

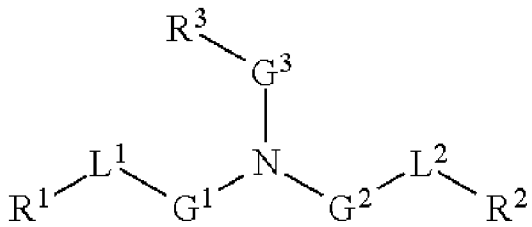
M_1 is -C(O)O-;

R_4 is $-(CH_2)_nOH$, and n is selected from 1, 2, 3, 4, or 5;

M is -OC(O)-; and

R_2 and R_3 are both C_{6-10} alkyl,

compounds of Formula (06-I):



(06-I)

wherein

L^1 and L^2 is -O(C=O)-;

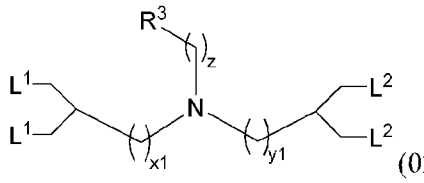
G^1 and G^2 are each independently unsubstituted C_4-C_8 alkylene;

G^3 is C_3-C_8 alkylene;

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^3 is H or OH,

Compounds of Formula (02-V-B)



(02-V-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alky;

R^3 is -OR^6;

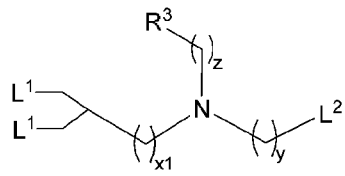
R^6 is hydrogen;

z is an integer from 2 to 12;

x1 is an integer from 0 to 9;

y1 is an integer from 0 to 9;

Compounds of Formula (02-VI-F)



(02-VI-B)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

R^3 is -OR^6;

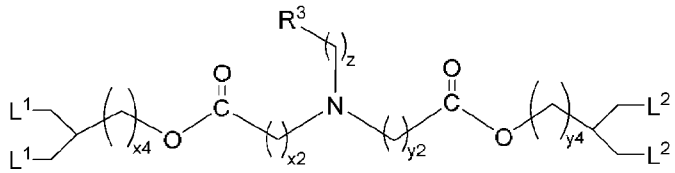
R^6 is hydrogen;

z is an integer from 2 to 12;

y is an integer from 2 to 12;

x1 is an integer from 2 to 5;

Compounds of Formula (02-V-F)



(02-V-F)

wherein

each L^1 is independently –OC(=O)R^1;

each L^2 is independently –OC(=O)R^2;

R^1 and R^2 are each independently C_6-C_{24} alkyl;

R^3 is -OR^6;

R^6 is hydrogen;

z is an integer from 2 to 12;

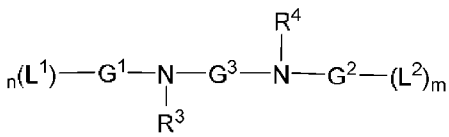
x2 is an integer from 2 to 9;

x4 is an integer from 0 to 3;

y2 is an integer from 2 to 9;

y4 is an integer from 0 to 3,

Compounds of formula (03-I)



(03-I),

wherein

G^1 and G^2 are each independently C_3-C_8 alkylene;

each L^1 is independently –OC(=O)R^1 or -C(=O)OR^1;

each L^2 is independently -C(=O)OR^2 or –OC(=O)R^2;

R^1 is independently C_6-C_{24} alkyl;

R^2 is independently C_6-C_{24} alkyl;

G^3 is C_2-C_{12} alkylene;

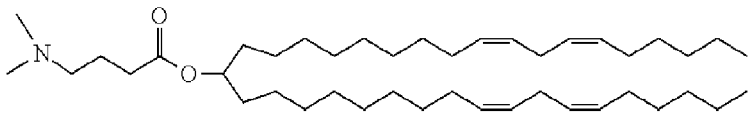
R^3 is C_3-C_8 cycloalkyl;

R^4 is C_1-C_4 hydroxylalkyl;

n is 1 or 2;

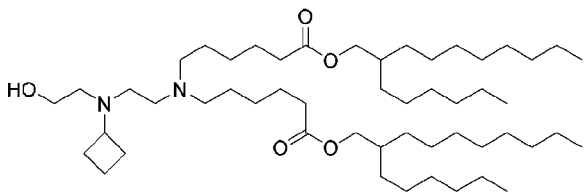
m is 1 or 2, and

compound 07-I:

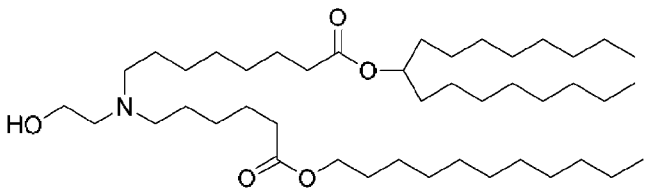


(compound 07-I).

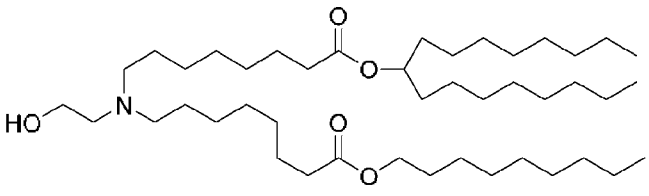
33.The LNP of any one of claims 28 to 30, wherein the ionizable lipid is selected from the following compounds:



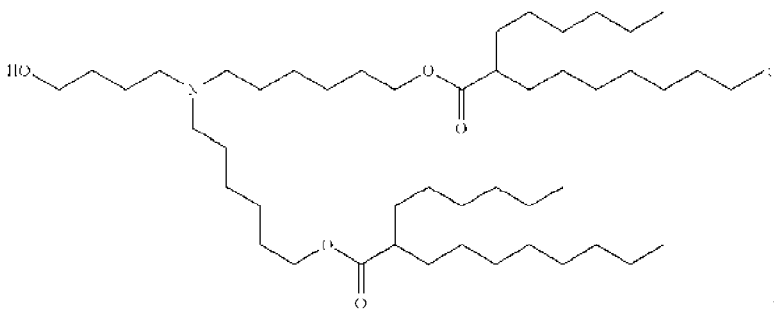
(Compound 01-1)



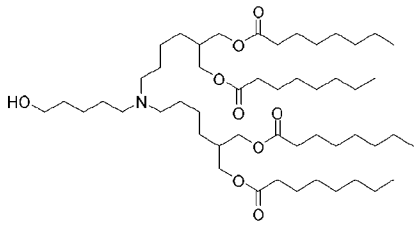
(SM102)



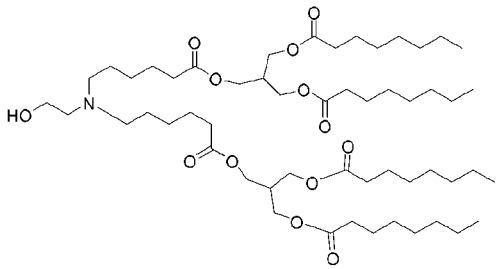
(Lipid 5)



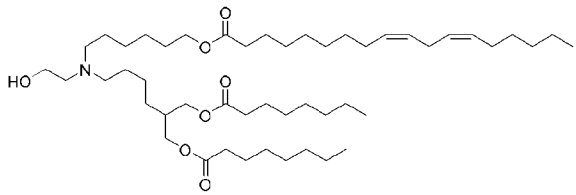
(Compound 06-1)



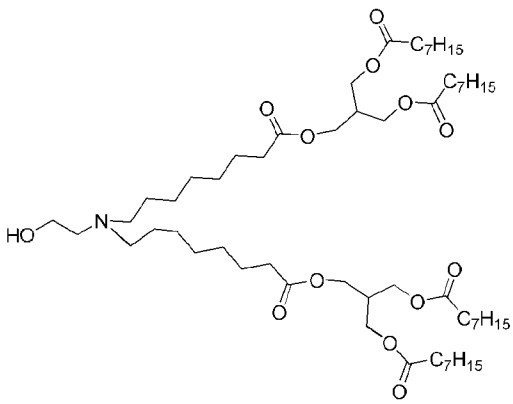
(Compound 02-3)



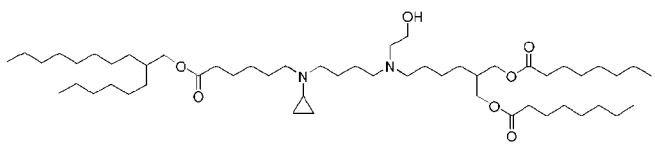
(Compound 02-6)



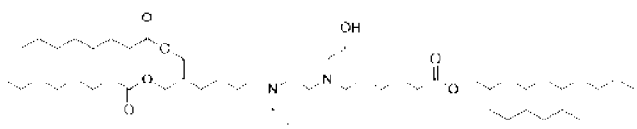
(Compound 02-22)



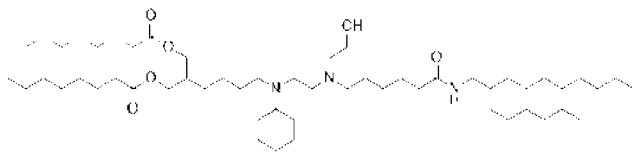
(Compound 02-65)



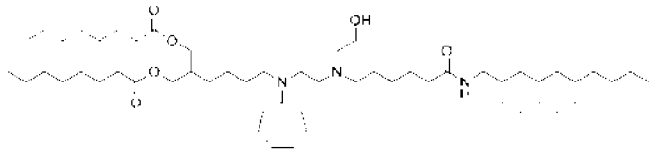
(Compound 03-190)



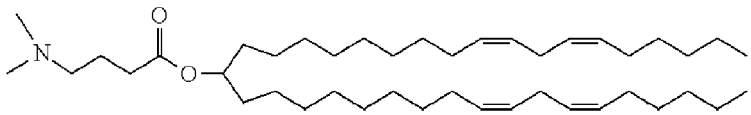
(Compound 03-135)



(Compound 03-140)



(Compound 03-143), and



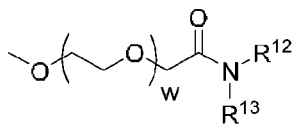
(Compound 07-I).

34.The LNP of any one of claims 27 to 33, further comprising a polymer conjugated lipid.

35.The LNP of claim 34, wherein the polymer conjugated lipid comprises from 0.5 to 5 mol%, preferably from 1 to 2 mol%, more preferably 1.5 mol% of the total amount of lipids in the LNP.

36.The LNP of claim 34 or 35, wherein the LNP has a molar ratio of the polymer conjugated lipid to the phospholipid of from 1:2 to 1:20, preferably from 1:3 to 1:18 or from 1:5 to 1:10.

37.The LNP of any one of claims 34 to 36, wherein the polymer conjugated lipid is a PEGylated lipid, preferably with the structure:



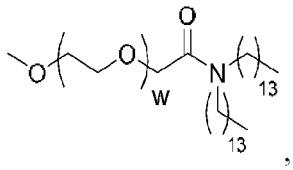
,

or a pharmaceutically acceptable salt thereof, wherein

R^{12} and R^{13} are each independently a straight or branched, alkyl or alkenyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and

w is an integer ranging from 30 to 60, preferably from 45 to 55, more preferably w is 49.

38.The LNP of any one of claims 34 to 36, wherein the polymer conjugated lipid is a PEGylated lipid with the structure:



or a pharmaceutically acceptable salt thereof, wherein

w is an integer ranging from 30 to 60, preferably from 45 to 55, more preferably w is 49preferably the polymer conjugated lipid is DMG-PEG or DMPE-PEG.

39.The LNP of any one of claims 27 to 38, further comprising a lipid stabilizer.

40. The LNP of claim 39, wherein the lipid stabilizer is selected from sterols, corticosteroids, tomatidine, tomatine, ursolic acid, and alpha-tocopherol, preferably selected from cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, prednisolone, dexamethasone, prednisone, hydrocortisone, tomatidine, tomatine, ursolic acid, and alpha-tocopherol.

41.The LNP of claim 39 or 40, wherein the LNP has a molar ratio of the lipid stabilizer to the phospholipid from 10:1 to 1:4, preferably from 5:1 to 1:3.

42.The LNP of any one of claims 39 to 41, wherein the lipid stabilizer comprises from 5 to 50 mol%, preferably from 8 to 40 mol%, more preferably from 10 to 30 mol% of a total amount of lipids in the LNP.

43.The LNP of any one of claims 27 to 42, wherein the phospholipid comprises from 1 to 30 mol%, preferably from 2 to 25 mol%, preferably from 3 to 20 mol%, more preferably from 5 to 15 mol% of a total amount of lipids in the LNP.

44.The LNP of any one of claims 27 to 43, wherein the LNP has a size of from 20 nm to 300 nm, preferably from 50 nm to 150 nm, preferably from 60 nm to 140 nm, more preferably from 80 nm to 100 nm, even more preferably from 85 nm to 95 nm, as determined using dynamic light scattering.

45.A composition comprising lipid nanoparticles (LNPs) and a therapeutic payload, wherein each LNP is an LNP of any one of claims 27 to 44.

46.The composition of claim 45, wherein at least 80%, preferably at least 85% of the LNPs encapsulate the therapeutic payload.

47.The composition of claim 45 or 46, wherein wherein the therapeutic payload is selected from nucleic acids, preferably selected from DNAs and RNAs, more preferably selected from

catalytic DNA, plasmid DNA, aptamer, complementary DNA, mRNA, antisense oligonucleotide, miRNA, miRNA inhibitor, micRNA, multivalent RNA, dsRNA, shRNA, antisense RNA, tRNA, aiRNA, a ribozyme, an aptamer, and a vector.

48. The composition of claim 45 or 46, wherein the therapeutic payload is a mRNA.

49.A method for expressing protein in a cell, comprising introducing the composition of claim 48, to the cell.

50.The method of claim 48, wherein the cell is a mammalian cell.

51.A method for delivering a protein to a subject, comprising administering the composition of claim 48 to the subject, wherein the mRNA encodes the protein.

52.The method of claim 51, wherein the composition is administered systemically.

53.The method of claim 51 or 52, wherein the subject is a mammal, in particular, a human.

FIGURES

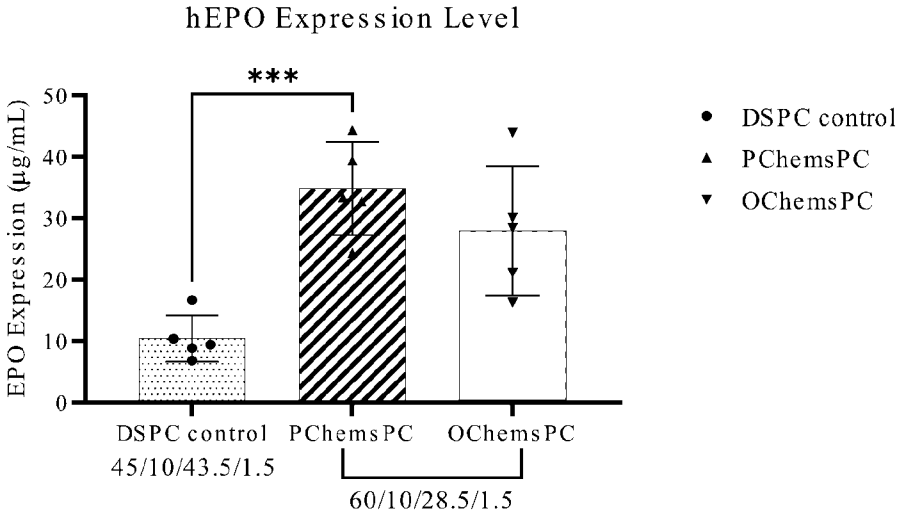


FIG.1

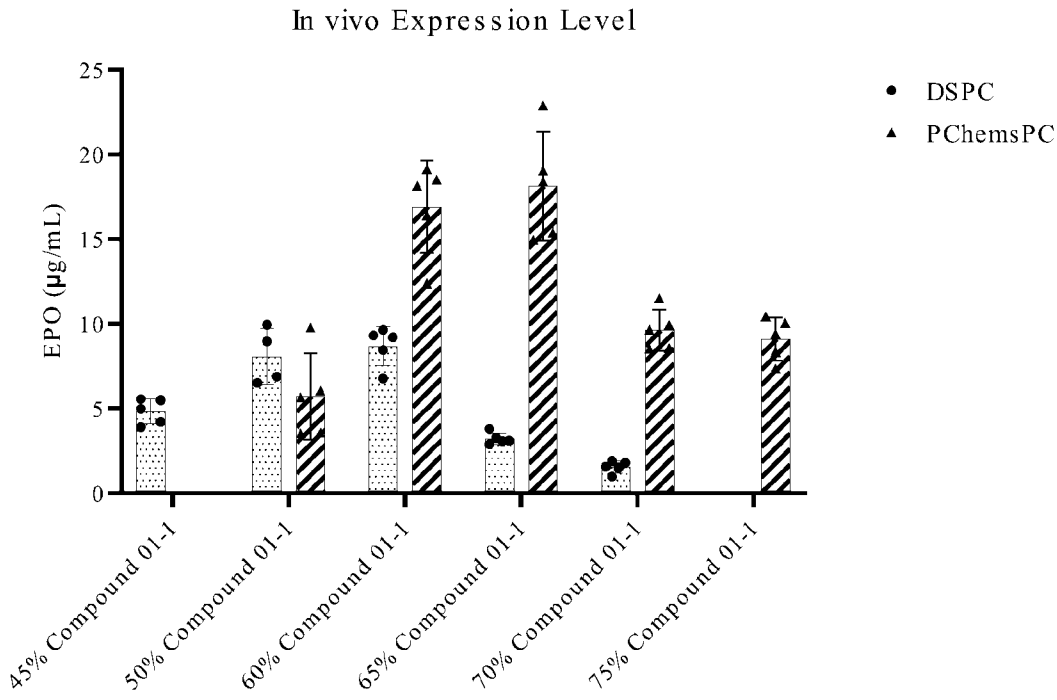


FIG.2

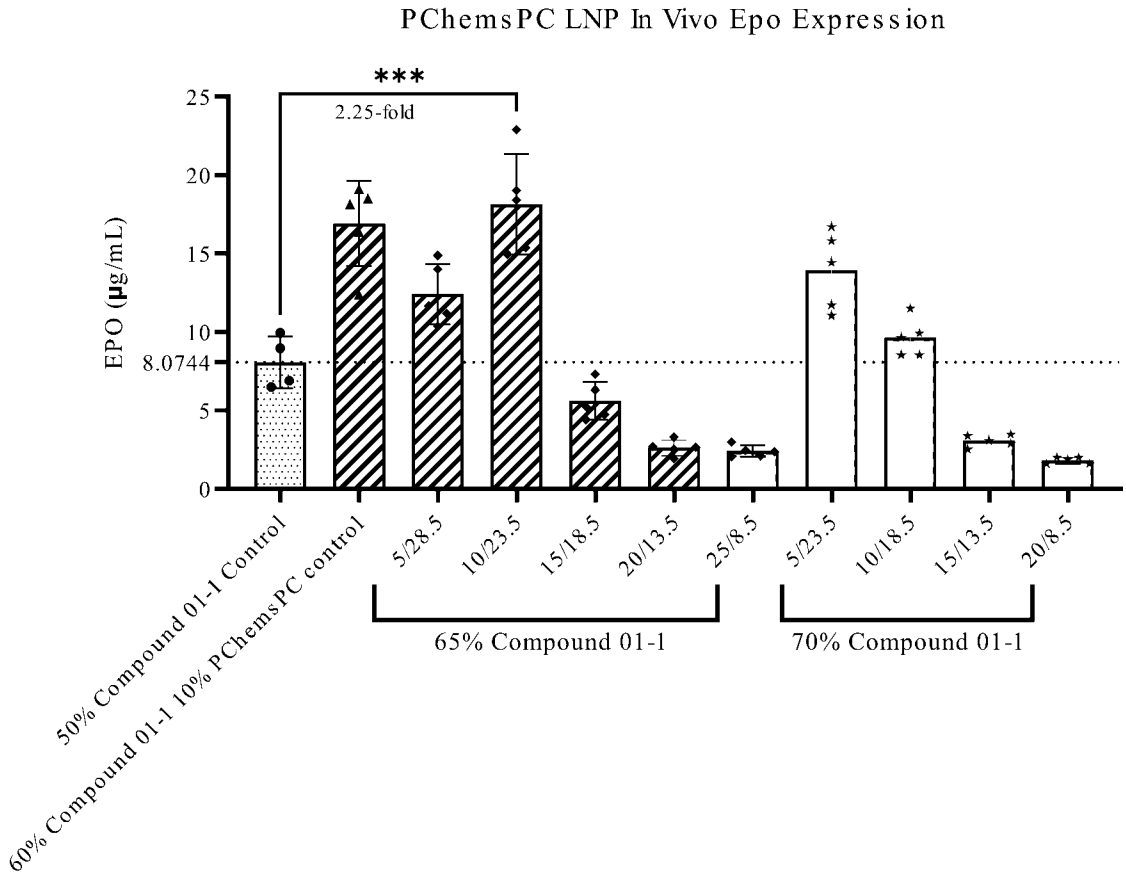

PChemsPC LNP In Vivo Epo Expression
***
2.25-fold
EPO (μg/mL)
25
20
15
10
8.0744
5
0
50% Compound 01-1 Control
60% Compound 01-1 10% PChemsPC control
5/28.5
10/23.5
15/18.5
20/13.5
25/8.5
5/23.5
10/18.5
15/13.5
20/8.5
65% Compound 01-1
70% Compound 01-1

FIG. 3

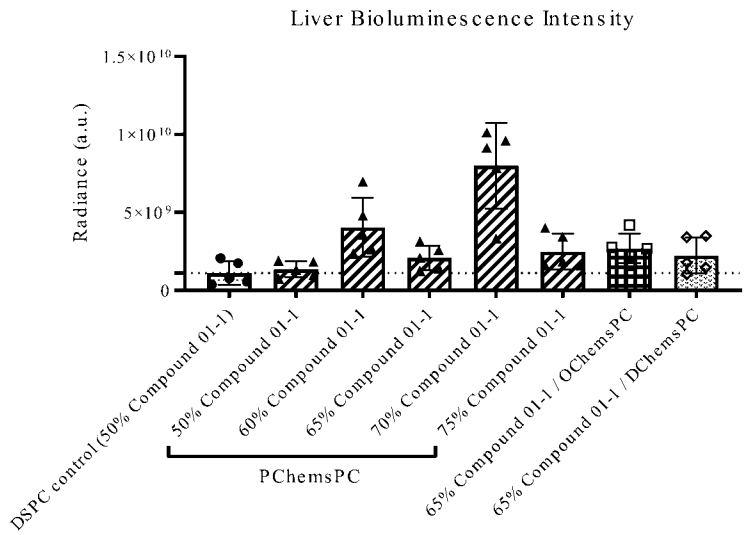

Liver Bioluminescence Intensity
Radiance (a.u.)
1.5×10^10
1×10^10
5×10^9
0
DSPC control (50% Compound 01-1)
50% Compound 01-1
60% Compound 01-1
65% Compound 01-1
70% Compound 01-1
75% Compound 01-1
65% Compound 01-1 / OChemsPC
65% Compound 01-1 / DChemsPC
PChemsPC

FIG.4

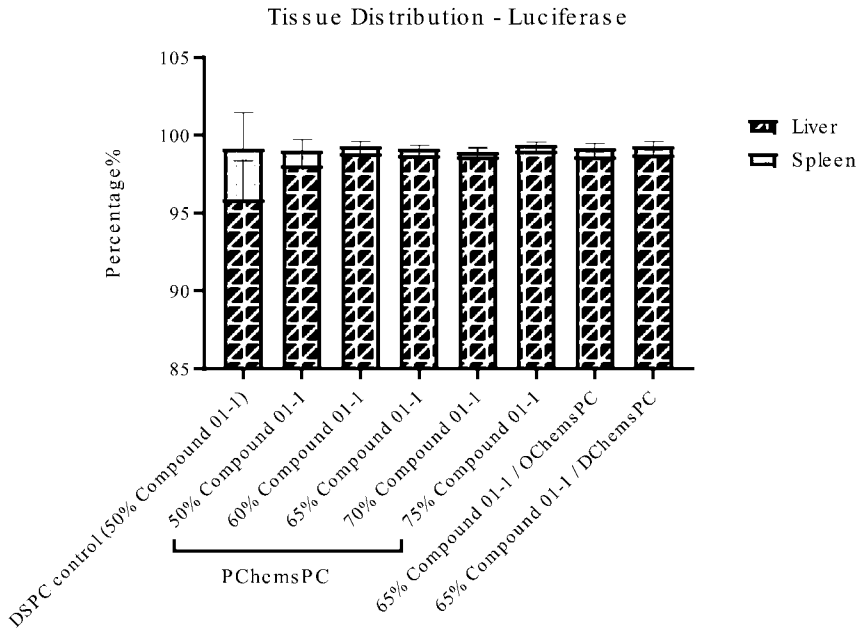

Tissue Distribution - Luciferase
Percentage%
105
100
95
90
85
Liver
Spleen
DSPC control (50% Compound 01-1)
50% Compound 01-1
60% Compound 01-1
65% Compound 01-1
70% Compound 01-1
75% Compound 01-1
65% Compound 01-1 / OChemsPC
65% Compound 01-1 / DChemsPC
PChemsPC

FIG.5

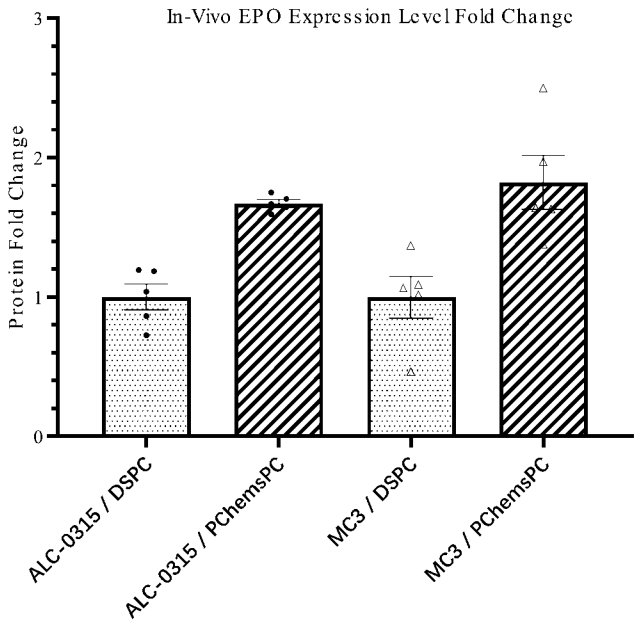

In-Vivo EPO Expression Level Fold Change
Protein Fold Change
3
2
1
0
ALC-0315 / DSPC
ALC-0315 / PChemsPC
MC3 / DSPC
MC3 / PChemsPC

FIG.6

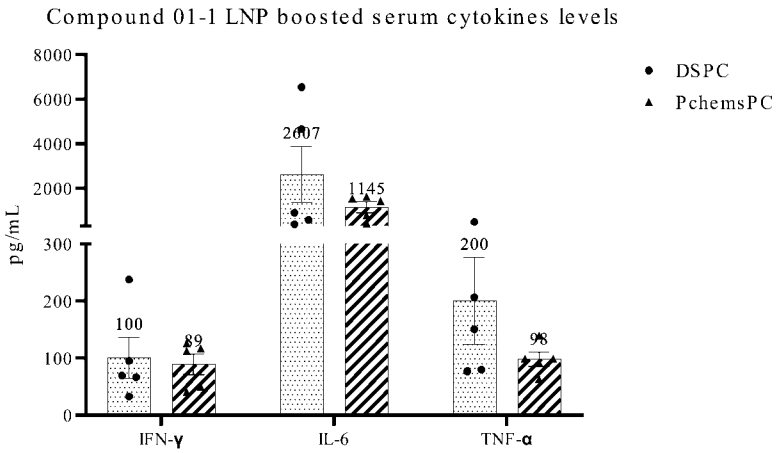


FIG.7

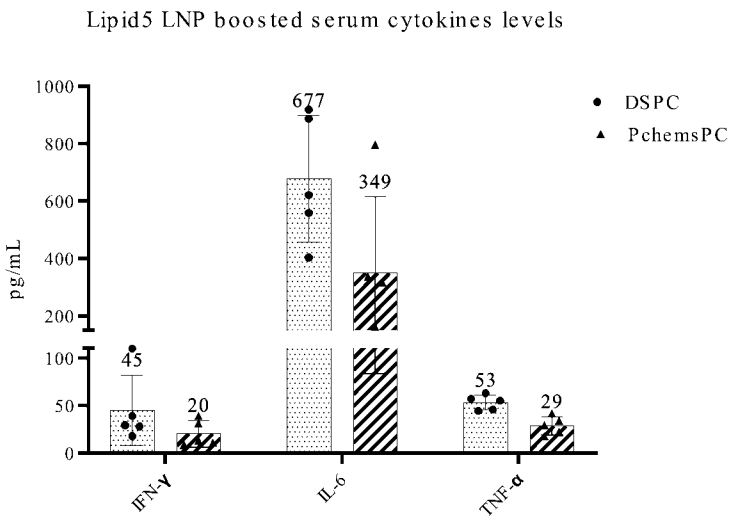


FIG.8

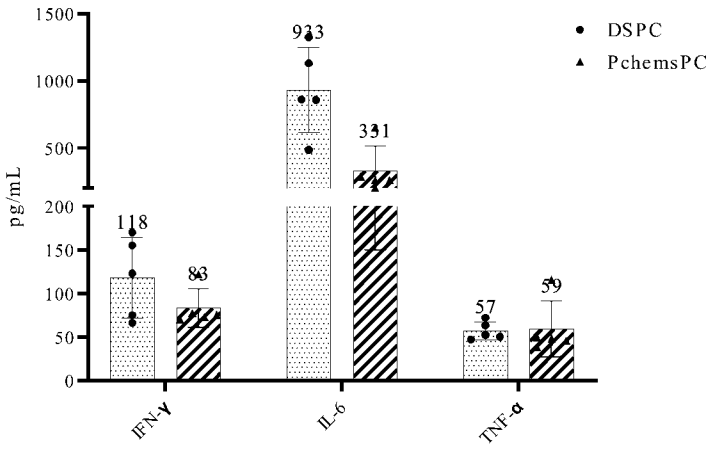


FIG.9

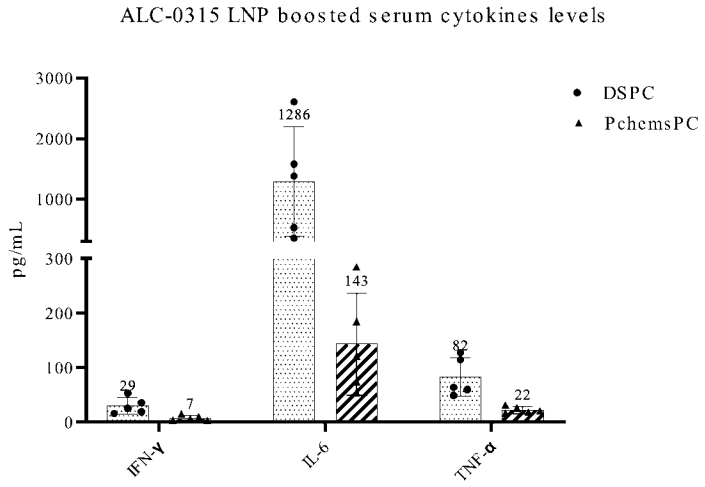


FIG.10

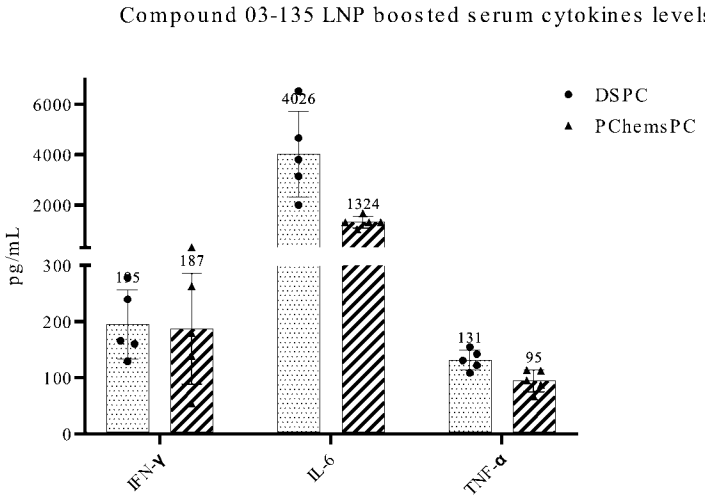


FIG.11

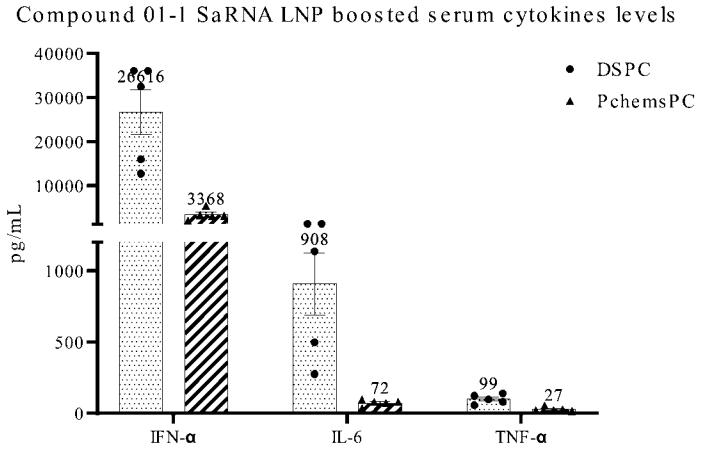


FIG. 12

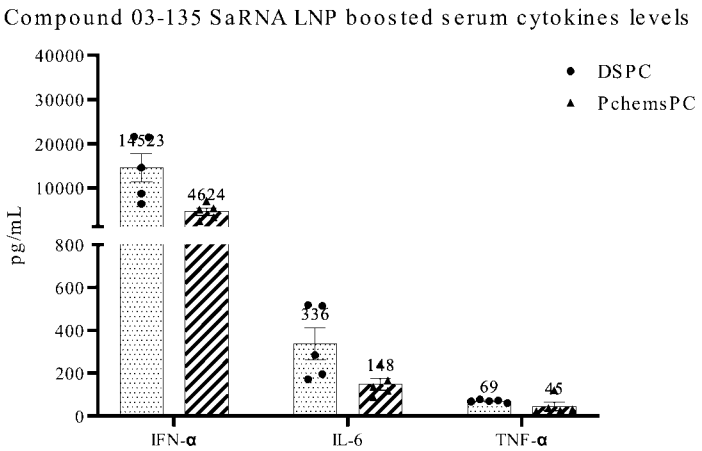

Compound 03-135 SaRNA LNP boosted serum cytokines levels
DSPC
PchemsPC
pg/mL
40000
30000
20000
10000
800
600
400
200
0
14523
4624
336
148
69
45
IFN-α
IL-6
TNF-α

FIG.13

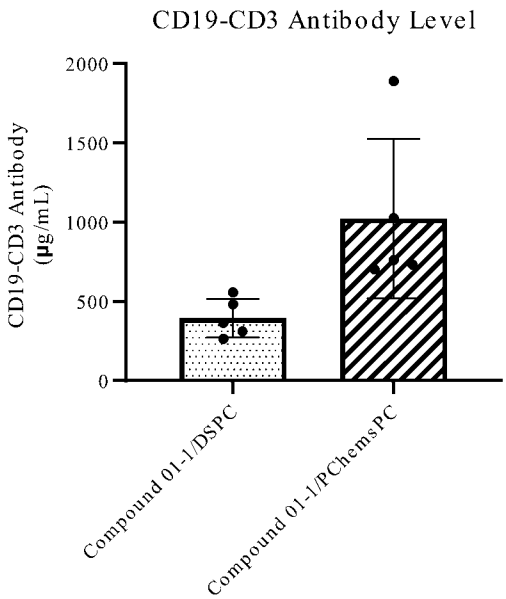

CD19-CD3 Antibody Level
CD19-CD3 Antibody (μg/mL)
2000
1500
1000
500
0
Compound 01-1/DSPC
Compound 01-1/PChemsPC

FIG.14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/140052

A. CLASSIFICATION OF SUBJECT MATTER

A61K9/127(2006.01)i; A61K9/51(2006.01)i

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)

IPC: A61K

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

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

CNABS, CNTXT,DWPI, WOTXT, EPTXT, USTXT, CNKI, PUBMED, ISI_Web of Science, Science Direct, STNext: lipid nanoparticle, LNP, sterol moiety, ionizable lipid, polymer conjugated lipid, structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2023031394 A1 (CUREVAC SE. et al.) 09 March 2023 (2023-03-09) claims 1-75	1-53
PX	WO 2023031392 A2 (CUREVAC SE. et al.) 09 March 2023 (2023-03-09) claims 1-46,examples 1-13	27-53
Y	WO 2021250263 A1 (ETHERNA IMMUNOTHERAPIES NV) 16 December 2021 (2021-12-16) claims 1-16, description page 7 line 1-page 11 line 28	1-53
Y	FLASIÑSKI, M. et al. "Sterol-Phospholipid Hybrids at the Air/Water Interface: Studies on Properties and Interactions with Parent Lipid Molecules" *Langmuir*, Vol. 32, 05 April 2016 (2016-04-05), pages 4095-4102	1-53
Y	CN 108368028 A (ACUITAS THERAPEUTICS, INC.) 03 August 2018 (2018-08-03) claims 1-40, description paragraph 0039	1-53
Y	CN 114026233 A (GENEVANT SCIENCES GMBH.) 08 February 2022 (2022-02-08) claims 1-82, description paragraphs 0008-0036	1-53

☑ 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2024	**03 April 2024**
Name and mailing address of the ISA/CN **CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	Authorized officer **JIAO,ShiYong** Telephone No. **(+86) 010-53961915**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/140052

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 9868691 B2 (MODERNATX, INC.) 16 January 2018 (2018-01-16) description column 6 line 45-description column 8 line 20	1-53
Y	WO 2010144740 A1 (ALNYLAM PHARMACEUTICALS, INC.) 16 December 2010 (2010-12-16) claims 1-30	1-53
Y	WO 2018081480 A1 (ACUITAS THERAPEUTICS, INC.) 03 May 2018 (2018-05-03) claims 1-90	1-53
Y	WO 2019089828 A1 (ACUITAS THERAPEUTICS, INC.) 09 May 2019 (2019-05-09) claims 1-71	1-53
Y	WO 2019141814 A1 (ETHERNA IMMUNOTHERAPIES NV.) 25 July 2019 (2019-07-25) claims 1-14	1-53
Y	WO 2020219941 A1 (GENEVANT SCIENCES GMBH.) 29 October 2020 (2020-10-29) claims 1-82	1-53
Y	WO 2021022173 A1 (MODERNATX, INC.) 04 February 2021 (2021-02-04) claims 1-160	1-53
Y	WO 2021030701 A1 (ACUITAS THERAPEUTICS, INC.) 18 February 2021 (2021-02-18) claims 1-66, examples 1-8	1-53
Y	WO 2022112855 A1 (GUANGZHOU RIBOBIO CO., LTD.) 02 June 2022 (2022-06-02) claims 1-46	1-53
Y	WO 2022152109 A2 (SUZHOU ABOGEN BIOSCIENCES CO., LTD.) 21 July 2022 (2022-07-21) claims 1-46	1-53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/140052

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.: **22-26, 49-53**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 22-23, 49-50 direct to a method for expressing protein in a cell; Claims 24-26, 51-53 direct to a method for delivering a protein to a subject. They do not meet the criteria set out in PCT Rules 39.1(iv). The search report has been made and based on the use of the composition of claims 21, 48 in manufacture of medicament for expressing protein in a cell or delivering a protein to a subject.

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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/140052

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2023031394 A1	09 March 2023	AU 2022336209 A1	18 January 2024
		IL 309505 A	01 February 2024
WO 2023031392 A2	09 March 2023	WO 2023031392 A3	13 April 2023
		CA 3230056 A1	09 March 2023
		IL 309502 A	01 February 2024
		AU 2022336664 A1	18 January 2024
WO 2021250263 A1	16 December 2021	BR 112022025217 A2	03 January 2023
		EP 4164596 A1	19 April 2023
		ZA 202300131 B	25 October 2023
		CA 3186776 A1	16 December 2021
		AU 2021286911 A1	09 February 2023
		MX 2022015690 A	22 February 2023
		IL 298765 A	01 February 2023
		JP 2023545886 A	01 November 2023
		KR 20230050313 A	14 April 2023
CN 108368028 A	03 August 2018	IL 286515 A	31 October 2021
		IL 286515 B1	01 October 2023
		IL 286515 B2	01 February 2024
		US 2020121809 A1	23 April 2020
		US 11712481 B2	01 August 2023
		PL 3368507 T3	27 March 2023
		DK 3368507 T3	27 February 2023
		HRP 20230209 T1	14 April 2023
		US 2019314524 A1	17 October 2019
		US 11040112 B2	22 June 2021
		US 2017119904 A1	04 May 2017
		US 10166298 B2	01 January 2019
		IL 258501 A	31 May 2018
		IL 258501 B	31 October 2021
		CA 3201644 A1	04 May 2017
		IL 307179 A	01 November 2023
		ES 2938557 T3	12 April 2023
		JP 2018533573 A	15 November 2018
		JP 7030690 B2	07 March 2022
		AU 2023274244 A1	21 December 2023
		WO 2017075531 A1	04 May 2017
		CA 3003055 A1	04 May 2017
		CA 3003055 C	01 August 2023
		HUE 061564 T2	28 July 2023
		US 2022072155 A1	10 March 2022
		US 11648324 B2	16 May 2023
		EP 4212510 A1	19 July 2023
		PT 3368507 T	07 February 2023
		AU 2016343803 A1	26 April 2018
		AU 2016343803 A8	07 June 2018
		AU 2016343803 B2	29 April 2021
		FI 3368507 T3	21 March 2023
		JP 2022081512 A	31 May 2022
		RS 63986 B1	31 March 2023
		LT 3368507 T	10 March 2023

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/140052

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				SI	3368507	T1	28 April 2023
				AU	2021206881	A1	19 August 2021
				AU	2021206881	B2	07 September 2023
				EP	3368507	A1	05 September 2018
				EP	3368507	B1	07 December 2022
CN	114026233	A	08 February 2022	AU	2020262437	A1	23 December 2021
				MA	55766	A	02 March 2022
				EP	3959314	A1	02 March 2022
				EP	3959314	A4	08 February 2023
				US	2022168222	A1	02 June 2022
				WO	2020219941	A1	29 October 2020
				CA	3137450	A1	29 October 2020
				JP	2022530018	A	27 June 2022
US	9868691	B2	16 January 2018	None			
WO	2010144740	A1	16 December 2010	EP	3431076	A1	23 January 2019
				EP	3431076	B1	06 October 2021
				PT	2440183	T	30 October 2018
				SG	10201912450	XA	30 March 2020
				US	2012183602	A1	19 July 2012
				US	8802644	B2	12 August 2014
				AU	2019204984	A1	01 August 2019
				AU	2019204984	B2	28 January 2021
				LT	2440183	T	10 August 2018
				KR	20200006176	A	17 January 2020
				KR	102205886	B1	21 January 2021
				JP	2015232048	A	24 December 2015
				JP	6132321	B2	24 May 2017
				KR	20170091798	A	09 August 2017
				KR	101987962	B1	11 June 2019
				JP	2018141019	A	13 September 2018
				JP	6592144	B2	16 October 2019
				MX	2011013320	A	28 February 2012
				EA	201791744	A2	30 March 2018
				EA	201791744	A3	31 July 2018
				CA	2764609	A1	16 December 2010
				CA	2764609	C	02 October 2018
				PL	3431076	T3	31 January 2022
				MX	342785	B	12 October 2016
				HUE	056773	T2	28 March 2022
				DK	3431076	T3	20 December 2021
				HK	1212620	A1	17 June 2016
				ES	2689168	T3	08 November 2018
				US	2015166465	A1	18 June 2015
				US	9394234	B2	19 July 2016
				PT	3431076	T	26 October 2021
				CY	1120641	T1	11 December 2019
				SI	3431076	T1	29 April 2022
				HRP	20181221	T1	05 October 2018
				NZ	596958	A	30 April 2014
				SG	10201403054	SA	30 October 2014

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/140052

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		JP	2017122126	A	13 July 2017
		JP	6359719	B2	18 July 2018
		IL	290077	A	01 March 2022
		PL	2440183	T3	31 January 2019
		DK	2440183	T3	01 October 2018
		JP	2012530059	A	29 November 2012
		JP	5819291	B2	24 November 2015
		CY	1124769	T1	25 November 2022
		EP	2440183	A1	18 April 2012
		EP	2440183	A4	27 February 2013
		EP	2440183	B1	18 July 2018
		TR	201811076	T4	27 August 2018
		NZ	622843	A	30 October 2015
		NZ	712719	A	31 March 2017
		LT	3431076	T	25 October 2021
		KR	20230098713	A	04 July 2023
		KR	20120081065	A	18 July 2012
		KR	101766408	B1	10 August 2017
		CA	3014827	A1	16 December 2010
		EA	201190306	A1	30 January 2013
		EA	024960	B1	30 November 2016
		HRP	20211619	T1	04 February 2022
		KR	20190065474	A	11 June 2019
		KR	102066189	B1	14 January 2020
		IL	244945	A0	31 May 2016
		IL	244945	B	30 June 2020
		AU	2021201228	A1	11 March 2021
		AU	2021201228	B2	07 July 2022
		KR	20210008938	A	25 January 2021
		KR	102374518	B1	16 March 2022
		IL	274826	A	30 July 2020
		EA	201690312	A1	31 August 2016
		EA	028860	B1	31 January 2018
		HUE	038796	T2	28 November 2018
		KR	20220038506	A	28 March 2022
		US	2010324120	A1	23 December 2010
		US	8158601	B2	17 April 2012
		SI	2440183	T1	30 October 2018
		AU	2017202702	A1	18 May 2017
		AU	2017202702	B2	02 May 2019
		AU	2010259984	A1	12 January 2012
		AU	2010259984	B2	09 March 2017
		MX	2019010340	A	14 October 2019
		SG	176786	A1	30 January 2012
		US	2017143631	A1	25 May 2017
		MX	367665	B	30 August 2019
		ES	2901627	T3	23 March 2022
		IL	216876	A0	29 February 2012
		IL	216876	A	21 April 2016
WO 2018081480	A1	03 May 2018	EP	3532103	A1	04 September 2019

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/140052

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2019089828 A1	09 May 2019	None	
WO 2019141814 A1	25 July 2019	EP 3740241 A1	25 November 2020
		CA 3088485 A1	25 July 2019
		US 2024016738 A1	18 January 2024
		US 2020345641 A1	05 November 2020
		US 11684577 B2	27 June 2023
		JP 2021511376 A	06 May 2021
		JP 7333563 B2	25 August 2023
WO 2020219941 A1	29 October 2020	None	
WO 2021022173 A1	04 February 2021	CA 3149386 A1	04 February 2021
		AU 2020319876 A1	24 February 2022
		EP 4003296 A1	01 June 2022
		JP 2022543773 A	14 October 2022
		US 2022280639 A1	08 September 2022
WO 2021030701 A1	18 February 2021	JP 2022544652 A	20 October 2022
		CO 2022002685 A2	19 April 2022
		KR 20220053599 A	29 April 2022
		IL 290477 A	01 April 2022
		CL 2022000351 A1	03 February 2023
		CR 20220108 A	27 May 2022
		US 2023097090 A1	30 March 2023
		BR 112022002708 A2	31 May 2022
		CA 3150458 A1	18 February 2021
		ECSP 22018209 A	29 July 2022
		AU 2020328596 A1	31 March 2022
		EP 4013385 A1	22 June 2022
		JOP 20220037 A1	30 January 2023
		MX 2022001720 A	11 March 2022
		PE 20220968 A1	10 June 2022
		DE 112020003843 T5	19 May 2022
		DOP 2022000038 A	31 January 2023
		GB 2600859 A	11 May 2022
		ES 2918001 A2	13 July 2022
WO 2022112855 A1	02 June 2022	None	
WO 2022152109 A2	21 July 2022	WO 2022152109 A3	25 August 2022
		TW 202229227 A	01 August 2022