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(54) Title: LIPIDS FOR NUCLEIC ACID DELIVERY

(57) Abstract: Provided herein are ionizable lipids, compositions comprising the ionizable lipids, and methods of making and using the same. The ionizable lipids provided herein can be formulated in lipid compositions for the delivery of macromolecules, such as nucleic acids, in vitro, ex vivo, or in vivo.

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LIPIDS FOR NUCLEIC ACID DELIVERY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application No. 63/395,625 filed on August 5, 2022, the content of which is incorporated herein by reference in its entirety, and the benefit of U.S. Provisional Patent Application No. 63/516,415 filed on July 28, 2023, the content of which is incorporated herein by reference in its entirety

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application is being filed with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled txt_TP109361WO1 SL.txt, created on July 31, 2023, and is 203,428 bytes in size. The information in electronic format of the Sequence Listing is incorporated by reference in its entirety.

BACKGROUND

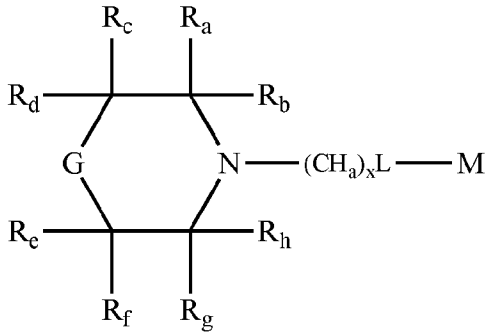
[0003] Transfection is the process of introducing nucleic acids into eukaryotic cells by non-viral methods. Transfection methods allow the introduction of negatively charged molecules (e.g. phosphate backbones of DNA and RNA) into cells having a negatively charged membrane. Chemicals such as calcium phosphate and DEAE-dextran, or cationic lipid-based reagents coat the DNA, neutralizing or even creating an overall positive charge to the molecule. The DNA-transfection reagent complex easily crosses the cell membrane, especially for lipids that have a "fusogenic" component, which enhances fusion with the lipid bilayer of the cell. With the recent advances in nucleic-acid based therapeutics, and continued need for transfection reagents with low toxicity, there is a continued need for novel ionizable lipids that can be used in vitro and in vivo.

[0004] Disclosed herein are compounds, compositions and methods that improve the efficiency of introducing macromolecules, such as nucleic acids, or small molecules (e.g, therapeutics), into cells. Compounds are provided, together with compositions containing these compounds and methods for using these new compounds and compositions for delivery of payloads, (e.g. a nucleic acid or small molecule), to cells. The compounds may be used alone for transfection, or they may be used in combination with additional reagents in transfection compositions. For example, the new compounds may be combined with one or more ionizable

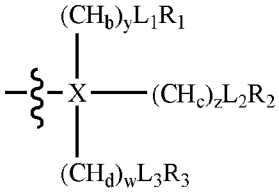
lipids and/or neutral lipids, with one or more cell surface ligands, with one or more fusion enhancing agents, and with one or more nuclear localization agents and one or more amphipathic peptides and any combinations thereof. The resulting compositions may be complexed with one or more macromolecules (e.g, nucleic acids, such as DNA or RNA, proteins, ribonucleoproteins, and the like) and used to deliver these macromolecules into cells.

BRIEF SUMMARY

[0005] In one aspect, a biodegradable compound is provided having the general structure (I):



where M is



(I)

G is O or a thiol-group selected from the group consisting of -S-, -SO-, -$S(O)_2$-, and -$S(CH_2)$-

L is selected from the group consisting of O, -C(=O)-, -$C(=O)N(R_4)$-, -$N(R_4)C(=O)$-, -$N(R_4)C(=O)N(R_5)$-, C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -$(R_6)P(=O)$- and a bond;

X is selected from the group consisting of C and N;

each R_a, R_b, R_c, R_d, R_e, R_f, R_g and R_h are independently selected from the group consisting of H, unbranched C_1-C_{12} alkyl groups, branched C_1-C_{12} alkyl groups, halogenated unbranched C_1-C_{12} alkyl groups, halogenated branched C_1-C_{12} alkyl groups, a C_3-C_7 membered alkyl ring, a C_3-C_7 carbocyclic ring formed from two R groups attached to an individual atom and a C_3-C_7 carbocyclic ring formed from R groups on two adjacent ring carbon atoms;

L_1, L_2 and L_3 are independently selected from the group consisting of O, -C(=O)-, -C(=O)N(R_6)-, -N(R_6)C(=O)-, -N(R_6)C(=O)N(R_7)-, -C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -(R_6)P(=O)- and a bond;

R_1, R_2 and R_3 are independently selected from the group consisting of H, unbranched C_1-C_{25} alkyl groups, branched C_1-C_{25} alkyl groups, halogenated unbranched C_1-C_{25} alkyl groups, and halogenated branched C_1-C_{25} alkyl groups;

each occurrence of R_4, R_5, R_6, and R_7 are independently selected from the group consisting of H and a C_1-$_6$ alkyl;

w, x, y and z are selected from the group of integers selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12;

-(CH_a)$_x$-, -(CH_b)$_y$-, -(CH_c)$_z$- and -(CH_d)$_w$- are selected from C_1-$_{12}$ alkyl, C_2-$_{12}$ alkene, and the absence of the methylene when w, x, y or z is 0; and

a, b, c, and d is 2 for C_1-$_{12}$ alkyl groups and selected from 1 and 2 for C_1-$_{12}$ alkene groups.

[0006] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **FIG. 1** is a graph depicting size (d.nm) and polydispersity index of the lipid-mRNA formulations.

[0008] **FIG. 2** is a graph depicting the percent encapsulation efficiency of the lipid-mRNA formulations.

[0009] **FIG. 3** is a graph depicting luciferase activity (in bioluminescence flux, photons/second (p/s)) in the liver of mice following intravenous administration of lipid-mRNA formulations.

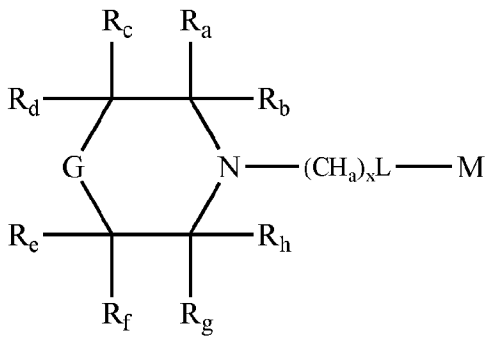
[0010] **FIG. 4** is a graph depicting the ratio of luciferase activity (in bioluminescence flux, photons/second (p/s)) in the liver by luciferase activity (in bioluminescence flux, photons/second (p/s)) in the spleen of mice following intravenous administration of lipid-mRNA formulations.

DETAILED DESCRIPTION

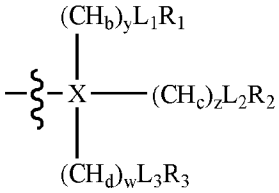
[0011] Lipid molecules are provided that are useful for improved methods of delivering payloads such as macromolecules, drugs and/or pharmaceutical agents, nutrients, or the like into eukaryotic cells, and that are particularly effective for delivery of wide variety of cells, tissues and organs, and provide a high efficiency of transfection. These lipid molecules are positively charged at about acidic pH, and advantageously can be used to prepare a complex with one or more neutral lipids and additional components such as fusogenic or fusion-enhancing molecules, additional cationic/ionizable lipids, cell surface ligands, cell adhesion molecules, nuclear localization agents and endosomal release agents, together with the payload (e.g., macromolecule or pharmaceutical agent, or nutrient, or the like). Such complexes are easily prepared and are stable and therefore are suitable for use in *in vitro*, *ex vivo* and *in vivo* applications, for example, delivery of therapeutic nucleic acids (e.g., siRNA therapeutics, mRNA vaccine preparations, and the like), in cell therapy applications (e.g., delivery of gene editing reagents), delivery of pharmaceutical agents, nutrients and the like to cells, e.g, in for cosmetic, nutraceutical, or therapeutic applications.

CATIONIC/IONIZABLE LIPIDS

[0012] **Item 1:** In one embodiment the biodegradable compound of the invention has the structure of compound I



where M is



(I)

G is O or a thiol-group selected from the group consisting of -S-, -SO-, -S(O)$_2$-, and -S(CH2)-

L is selected from the group consisting of O, -C(=O)-, -C(=O)N(R$_4$)-, -N(R$_4$)C(=O)-, -N(R$_4$)C(=O)N(R$_5$)-, C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -(R$_6$)P(=O)- and a bond;

X is selected from the group consisting of C and N;

each R$_a$, R$_b$, R$_c$, R$_d$, R$_e$, R$_f$, R$_g$ and R$_h$ are independently selected from the group consisting of H, unbranched C$_1$-C$_{12}$ alkyl groups, branched C$_1$-C$_{12}$ alkyl groups, halogenated unbranched C$_1$-C$_{12}$ alkyl groups, halogenated branched C$_1$-C$_{12}$ alkyl groups, a C$_3$-C$_7$ membered alkyl ring, a C$_3$-C$_7$ carbocyclic ring formed from two R groups attached to an individual atom and a C$_3$-C$_7$ carbocyclic ring formed from R groups on two adjacent ring carbon atoms;

L$_1$, L$_2$ and L$_3$ are independently selected from the group consisting of O, -C(=O)-, -C(=O)N(R$_6$)-, -N(R$_6$)C(=O)-, -N(R$_6$)C(=O)N(R$_7$)-, C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -(R$_6$)P(=O)- and a bond;

R$_1$, R$_2$ and R$_3$ are independently selected from the group consisting of H, unbranched C$_1$-$_{25}$ alkyl groups, branched C$_1$-C$_{25}$ alkyl groups, halogenated unbranched C$_1$-C$_{25}$ alkyl groups, and halogenated branched C$_1$-C$_{25}$ alkyl groups;

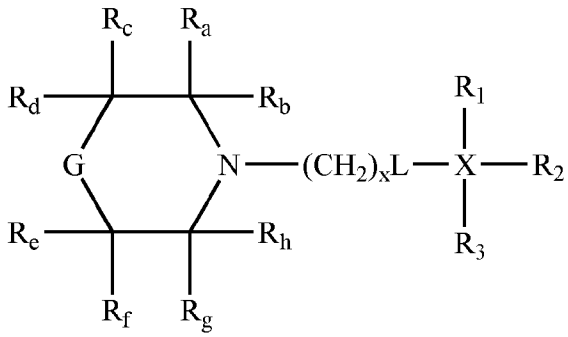
each occurrence of R$_4$, R$_5$, R$_6$, and R$_7$ are independently selected from the group consisting of H and a C$_1$-C$_6$ alkyl;

w, x, y and z are selected from the group of integers selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12;

$-(CH_a)_x-$, $-(CH_b)_y-$, $-(CH_c)_z-$ and $-(CH_d)_w-$ are selected from C_1-C_{12} alkyl, C_2-C_{12} alkene, and the absence of the methylene when w, x, y or z is 0; and

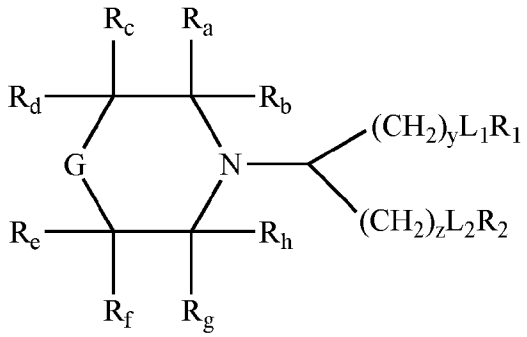
a, b, c, and d is 2 for C_1-C_{12} alkyl groups and selected from 1 and 2 for C_1-C_{12} alkene groups.

[0013] Item 2: In some embodiments the compound has the structure of compound I-A, wherein y, z and w are 0, L_1, L_2 and L_3 are a bond



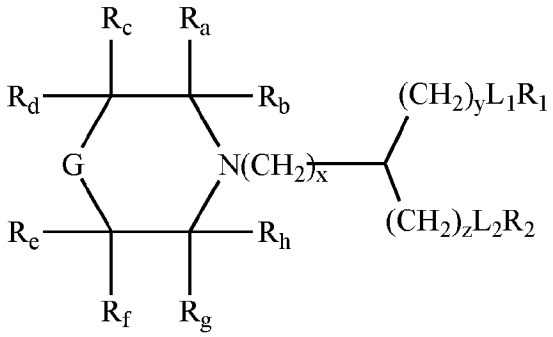
I-A.

[0014] Item 3: In certain embodiments the compound has the structure of compound I-B, wherein X is carbon, x and w are 0, L_3 is a bond, R_3 is H, and L is a bond



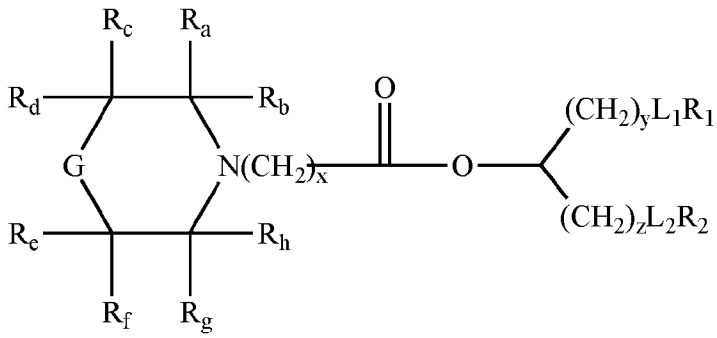
(I-B).

[0015] Item 4: Also provided are compounds having the structure I-C, wherein X=C, w=0, L_3 is a bond, R_3 is H, and L is a bond



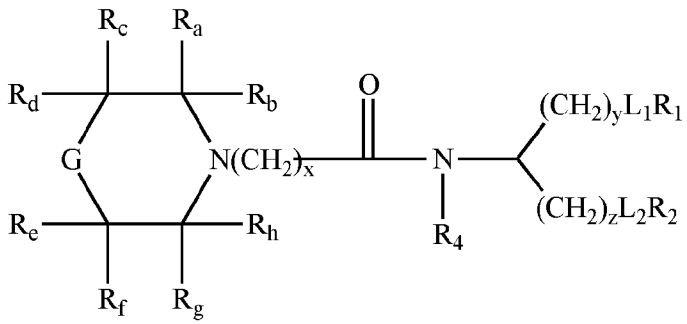
I-C.

[0016] Item 5: In some embodiments are compounds having the structure I-D wherein X=C, w=0, L_3 is a bond, R_3 is H, and L is -C(=O)O-



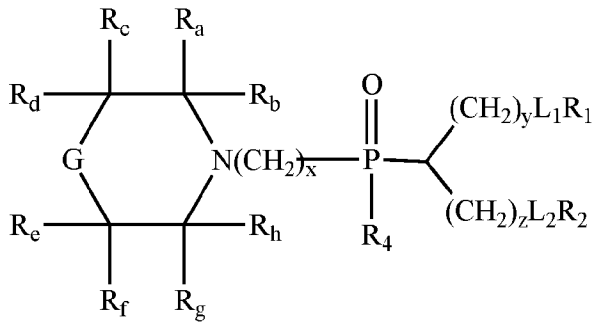
I-D.

[0017] Item 6: In certain embodiments are compounds having the structure I-E wherein X is Carbon, w is 0, L_3 is a bond, R_3 is H, and L is -C(=O)N(R_4)-



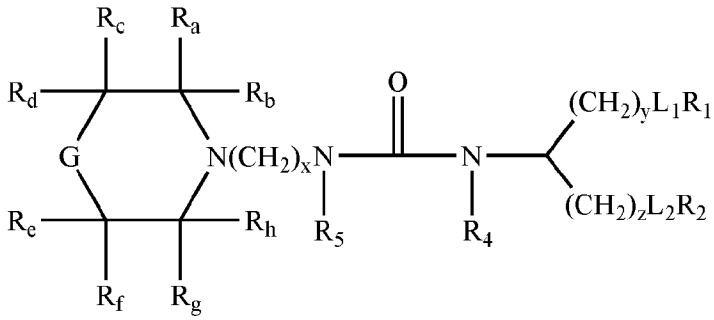
I-E.

[0018] Item 7: Also provided are compounds having the structure I-F, wherein X=C, w=0, L_3 is a bond, R_3 is H, and L is -(R_4)P(=O)-



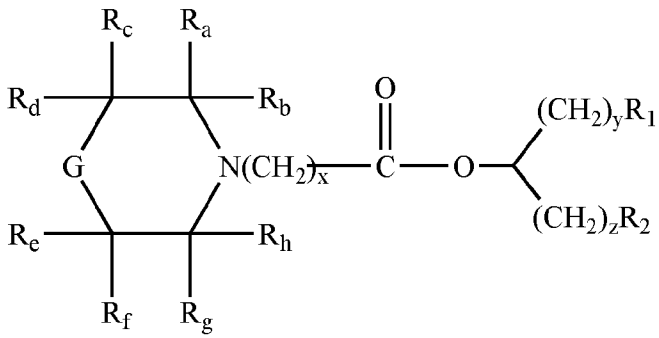
I-F.

[0019] Item 8: In some embodiments are compounds having the structure I-G, wherein X=C, w=0, L_3 is a bond, R_3 is H, and L is -(R_4)NC(=O)(R_5)N-



I-G.

[0020] Item 9: In certain embodiments are compounds having the structure I-H wherein X is C, L is -C(=O)O-, L_1, L_2 and L_3 are each a bond, R_3 is H, and w is 0

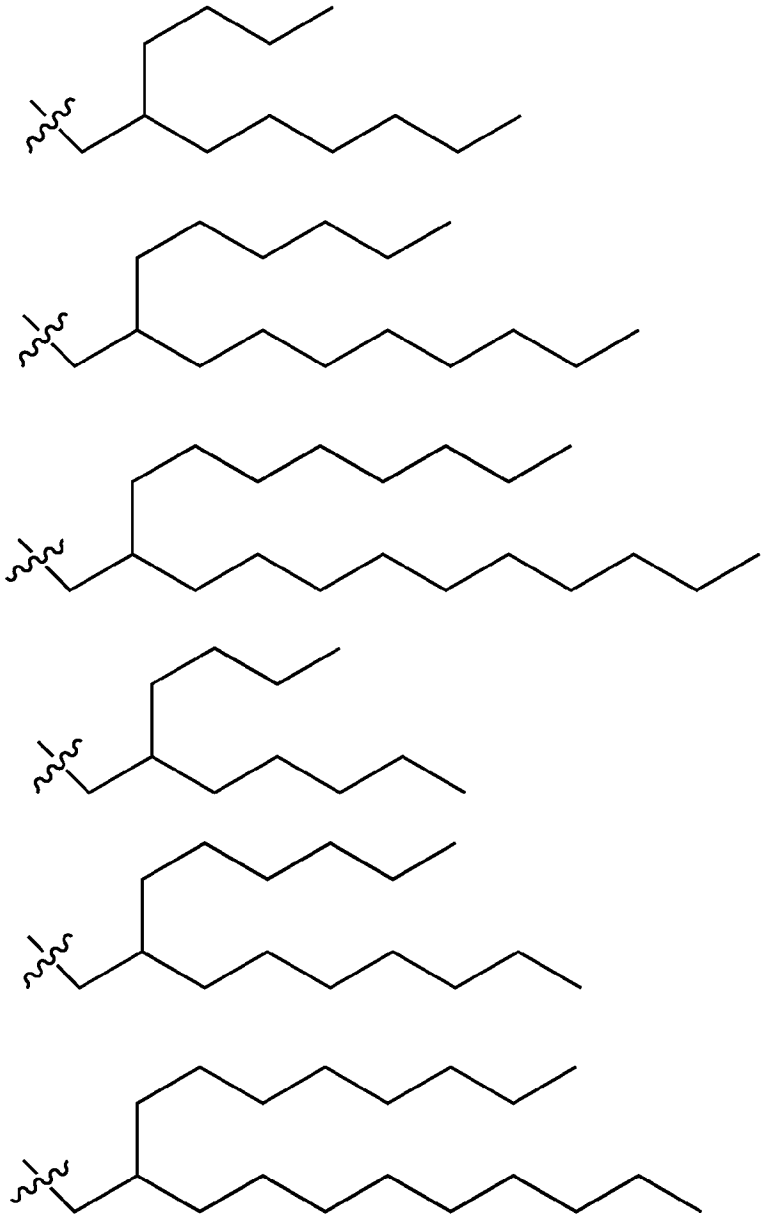


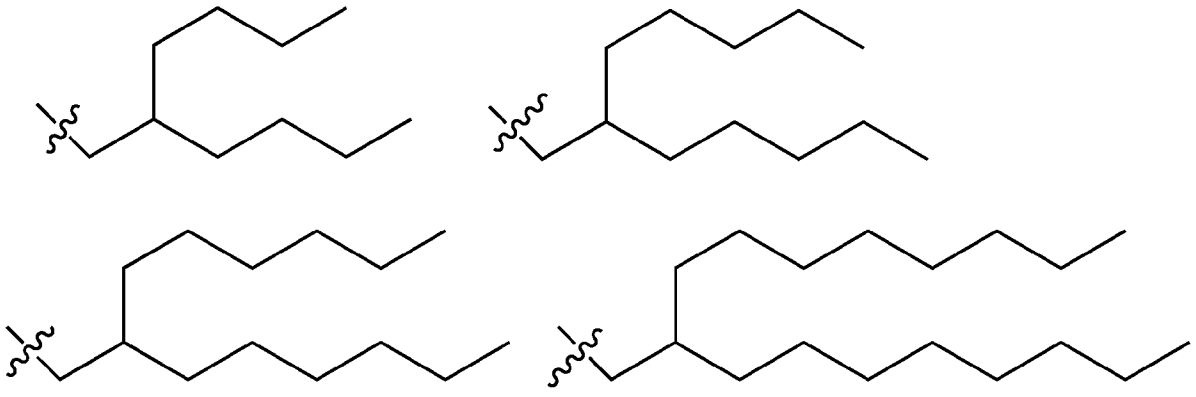
I-H.

[0021] Item 10: Also provided are compounds of compound I, wherein at each occurrence a halogen atom is selected from F, Cl, Br and I.

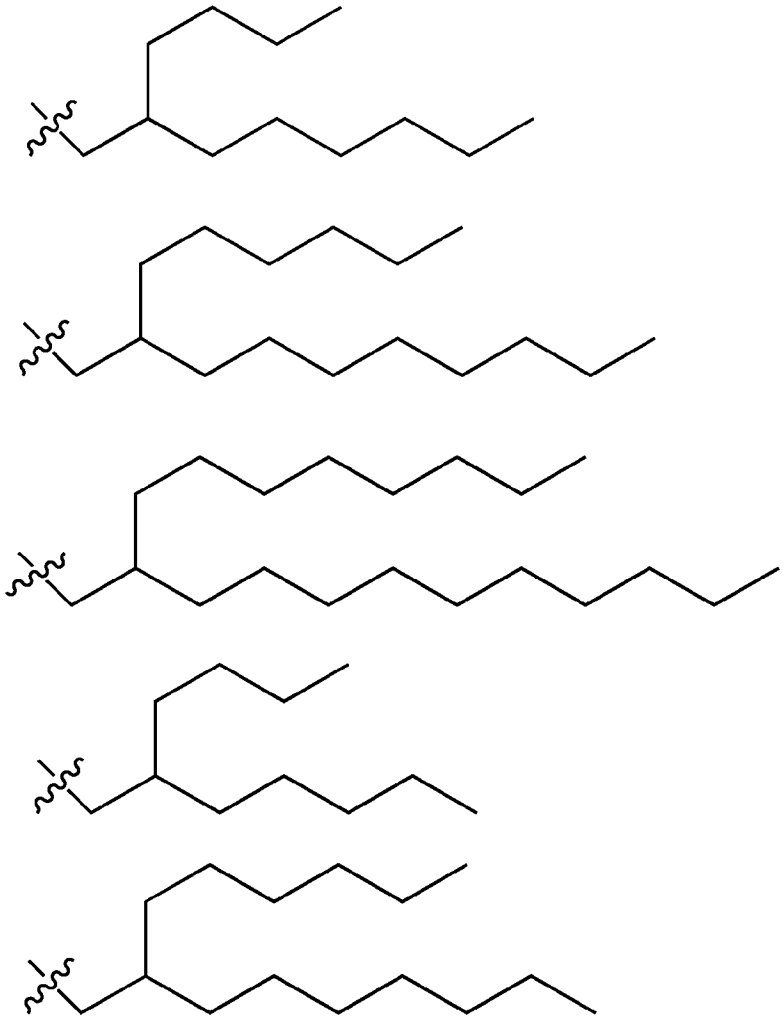
[0022] **Item 11:** In some embodiments of compound I, each of R_1, R_2 and R_3 are independently selected from the group consisting of H, a diene and a triene.

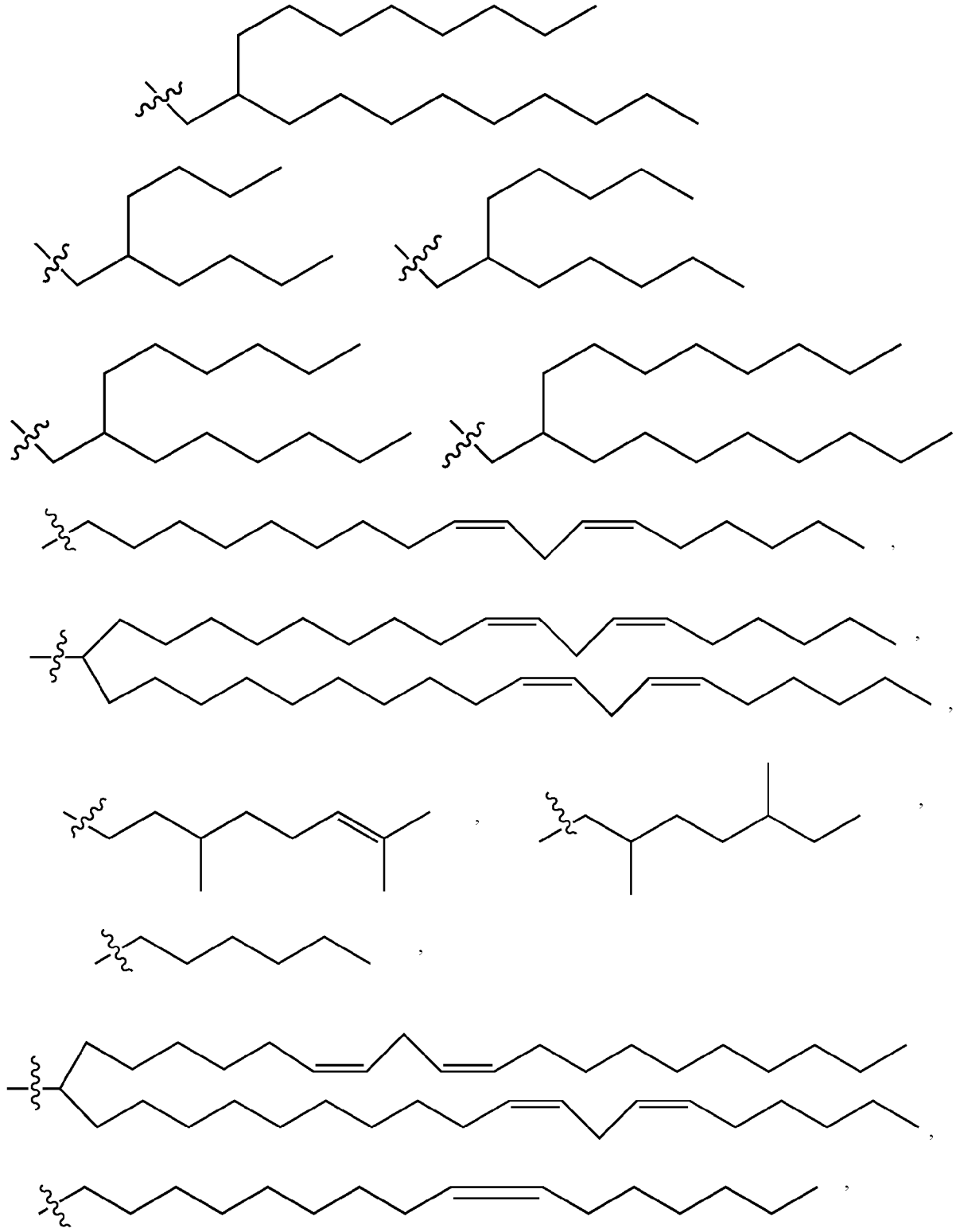
[0023] **Item 12:** In some embodiments of compound I, M has a structure selected from the group consisting of

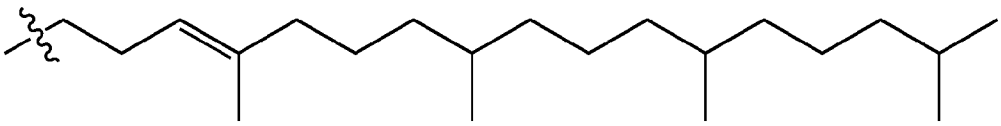




[0024] Item 13: In certain embodiments, each R_1, R_2 and R_3 of compound I is independently selected from the group consisting of H,







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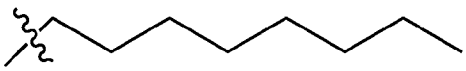
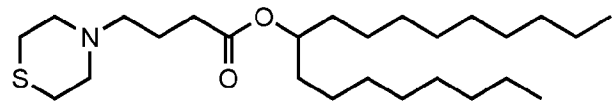


[0025] **Item 14:** Also provided are compounds having a structure selected from the group consisting of



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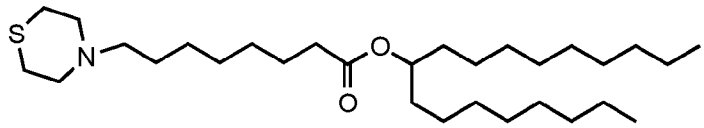

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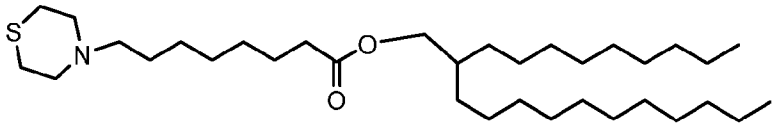

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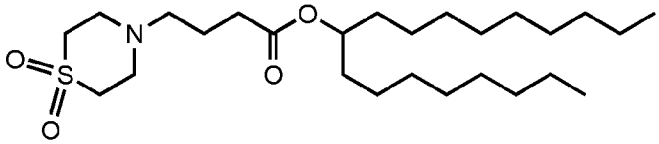

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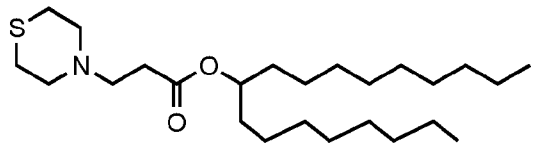

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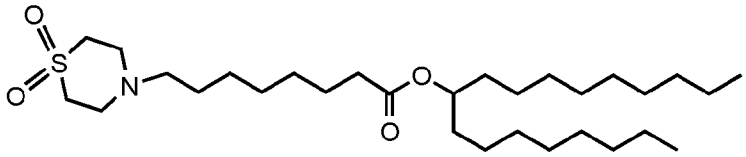

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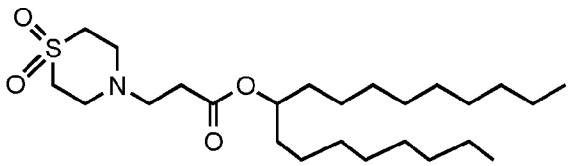
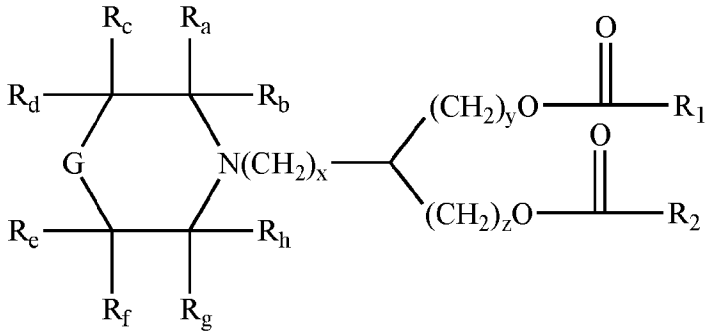
and



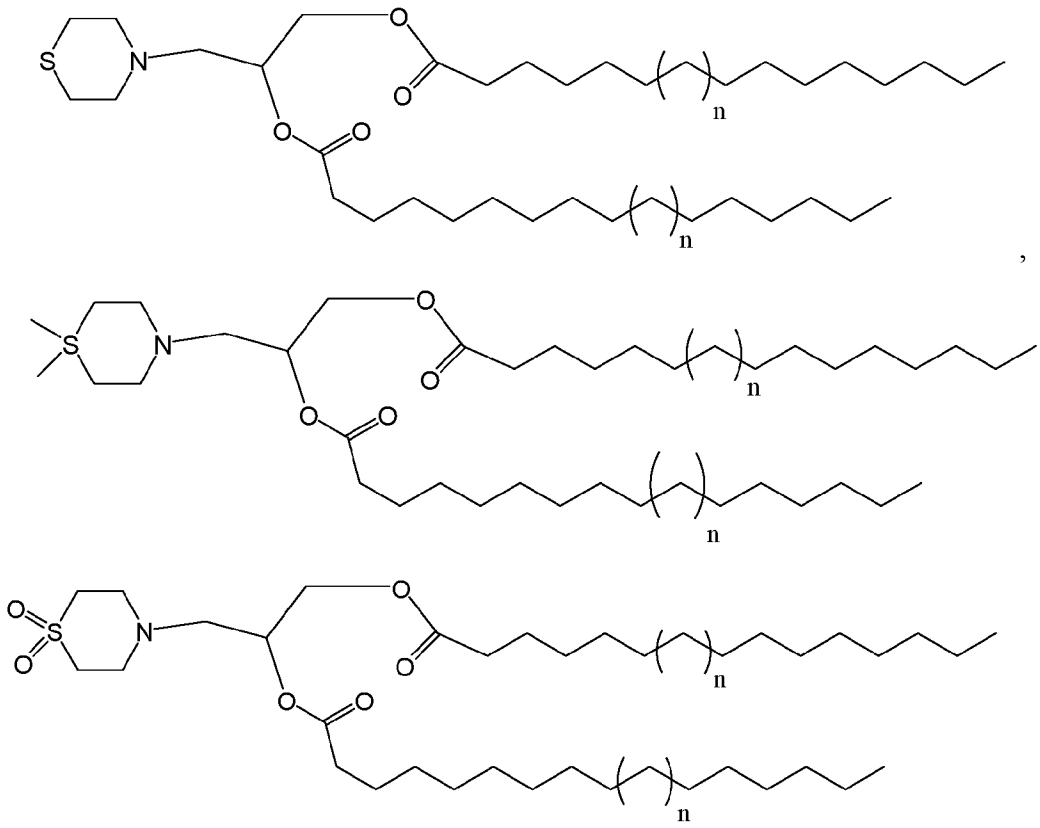
7.

[0026] **Item 15:** In some embodiments, compounds having the structure I-I, wherein X is C, L is a bond, L_1 and L_2 are both –OC(=O) and each L_1R_1 and L_2R_2 independently form an esterified fatty acid moiety, w is 0, L_3R_3 are a bond and H,



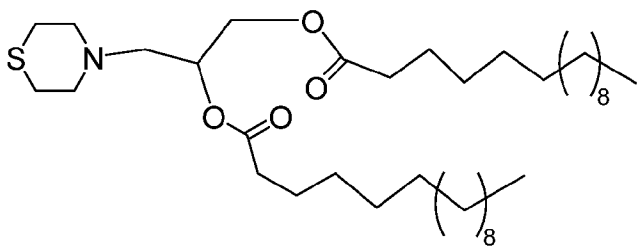
I-I.

[0027] **Item 16:** Also provided are compounds having a structure selected from the group consisting of



and

[0028] **Item 16a**. Also provided are compounds having a structure selected from the group consisting of



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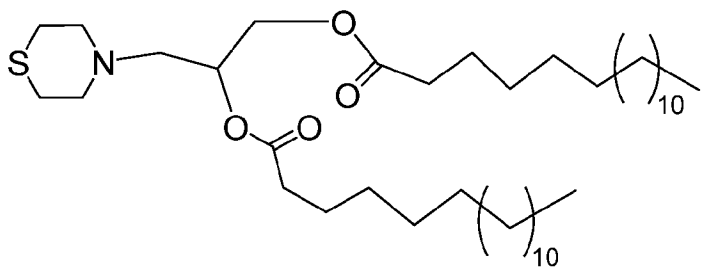

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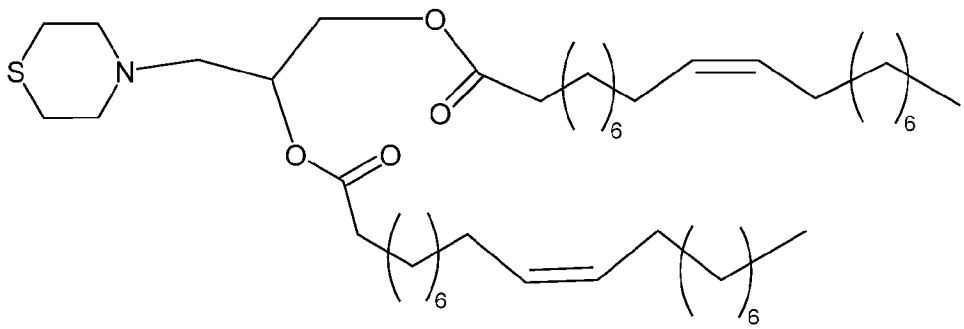

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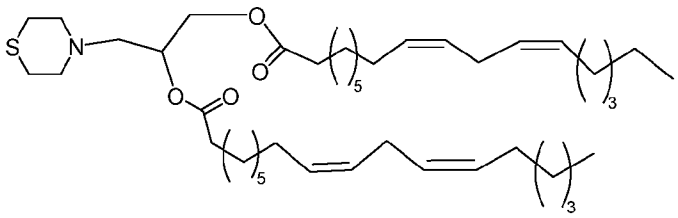

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[0029] **Item 17:** In some aspects compounds of I-I are provided in which one of L_1R_1 and L_2R_2 is an unsaturated fatty acid.

[0030] **Item 18:** In certain embodiments compounds of I-I are provided in which both L_1R_1, and L_2R_2, are an unsaturated fatty acid moiety.

[0031] **Item 19:** Also provided are compounds of I-I wherein the unsaturated fatty acid moiety is selected from the group consisting of α-linolenic acid (C 18:3), stearidonic acid (C 18:4),

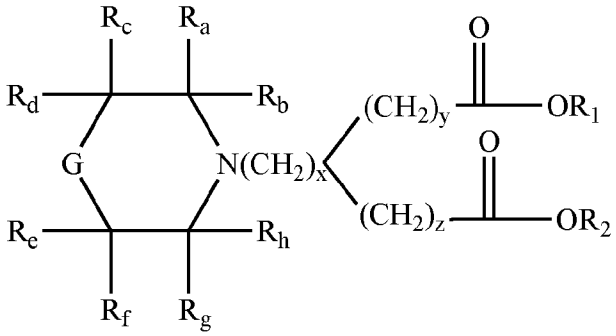
eicosapentaenoic acid (C 20:5), cervonic acid (C 22:6), linoleic acid (C 18:2), linolelaidic acid (C 18:2), γ-linolenic acid (C 18:3), dihomo-γ-linolenic acid (C 20:3), arachidonic acid (C 20:4), docosatetraenoic acid (C 22:4), palmitoleic acid (C 16:1), vaccenic acid (C 18:1), paullinic acid (C 20:1), oleic acid (C 18:1), elaidic acid (C 18:1), gondoic acid (C 20:1), erucic acid (C 22:1), nervonic acid (C 24:1), and mead acid (C 20:3).

[0032] Item 20: In some embodiments one of L_1R_1, and L_2R_2, is a saturated fatty acid moiety.

[0033] Item 21: In certain embodiments both of L_1R_1, and L_2R_2, are saturated fatty acid moiety.

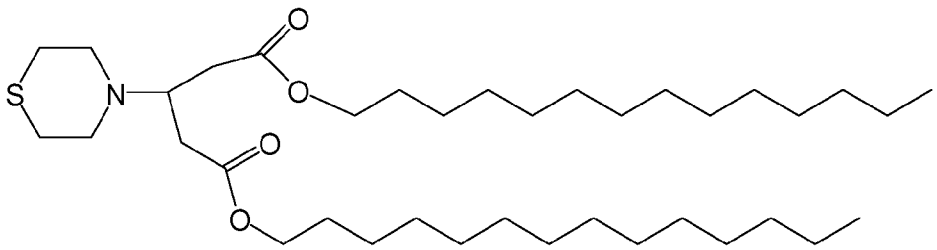
[0034] Item 22: Also provided are compounds in which the saturated fatty acid moiety is selected from the group consisting of propionic acid (C 3:0), butyric acid (C 4:0), valeric acid (C 5:0), caproic acid (C 6:0), enanthic acid (C 7:0), caprylic acid (C 8:0), pelargonic acid (C 9:0), capric acid (C 10:0), undecylic acid (C 11:0), lauric acid (C 12:0), tridecylic acid (C 13:0), myristic acid (C 14:0), pentadecylic acid (C 15:0), palmitic acid (C 16:0), margaric acid (C 17:0), stearic acid (C 18:0), nonadecylic acid (C 19:0), arachidic acid (C 20:0), heneicosylic acid (C 21:0), behenic acid (C 22:0), tyricosylic acid (C 23:0), lignoceric acid (C 24:0) and pentacosylic acid (C 25:0).

[0035] Item 23: In some aspects, the compound may have has the structure of I-K, where L is a bond and L_1R_1 and L_2R_2 form the esters $-C(=O)OR_1$, and $-C(=O)OR_2$



I K.

[0036] **Item 24:** In certain embodiments the compound may be selected from the group consisting of



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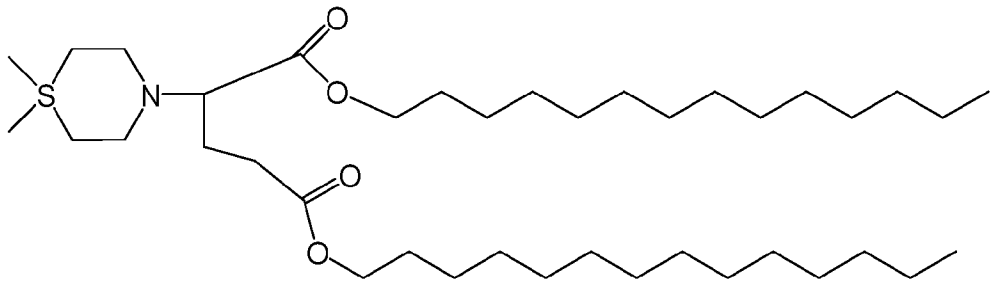

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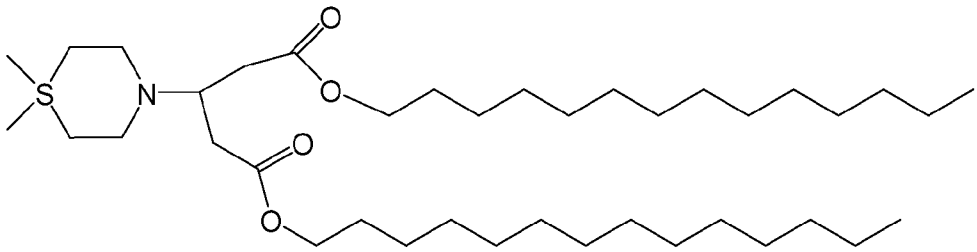

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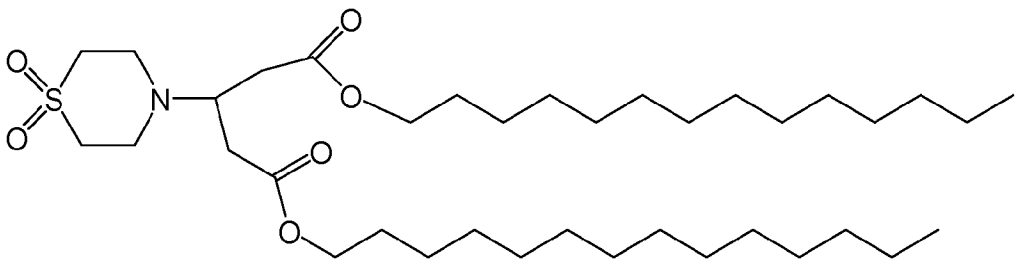
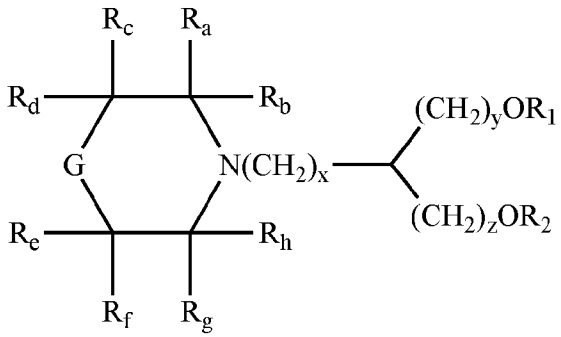
and



15.

[0037] Item 25: Also provided are compounds of structure I-I where the ester moieties -OR_1 and –OR_2 may be derived from an alcohol selected from the group consisting of tert-butyl alcohol (C4), tert-amyl alcohol (C 5), 3-methyl-3-pentanol (C 6), 1-hexanol (C 6), 1-heptanol (C 7), 1-octanol (C 8), 1-nonanol (C 9), 1-decanol (C 10), 1-undecanol (C 11), 1-dodecanol (C 12), 1-tridecanol (C 13), 1-tetradecanol (C 14), 1-pentadecanol (C 15), 1-hexadecanol (C 16), cis-9-hexadecen-1-ol (C 17), 1-n-heptadecanol (C 18), 1-octadecanol (C 19), 1-octadecenol (C 20), 1-nonadecanol (C 21) and 1-eicosanol (C 22).

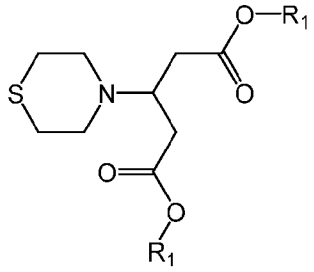
[0038] Item 26: In some embodiments, the compound may have the structure of I-L, where L is a bond, and L_1R_1 and L_2R_2 form ethers -OR_1 and -OR_2.

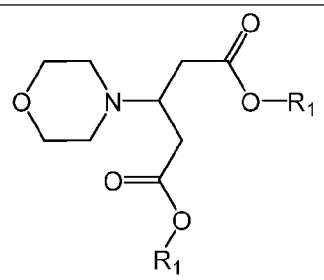
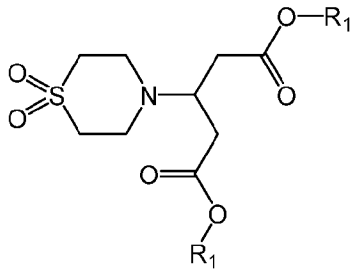


I-L.

[0039] Item 27: In certain embodiments R_1 and R_2 may be selected from the group consisting of C_1-C_{22} alkyl and C_2-C_{22} alkene groups.

[0040] Item 27a: In some embodiments, the compound may have the structure of any compound in the following table:

Compound Formula	R_1 moieties
 (II)	R_1 = Myristyl ($C_{14}H_{29}$) R_1 = Palmitoyl ($C_{16}H_{33}$) R_1 = Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9) R_1 = Oleyl ($C_{18}H_{37}$, cis-Δ^9) R_1 = Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12})

 (III)	R_1 = Myristyl ($C_{14}H_{29}$) R_1 = Palmitoyl ($C_{16}H_{33}$) R_1 = Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9) R_1 = Oleyl ($C_{18}H_{37}$, cis-Δ^9) R_1 = Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12})
 (IV)	R_1 = Myristyl ($C_{14}H_{29}$) R_1 = Palmitoyl ($C_{16}H_{33}$) R_1 = Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9) R_1 = Oleyl ($C_{18}H_{37}$, cis-Δ^9) R_1 = Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12})

[0041] Item 28: Certain embodiments provide for a composition comprising the compound of any one of items 1-27a and a payload.

[0042] Item 29: In certain embodiments the composition of item 28, further comprises one or more ionizable lipids.

[0043] Item 30: In certain embodiments the composition of item 28 or 29, further comprises a helper lipid.

[0044] Item 31: In certain embodiments the composition of any one of items 28-30, further comprises a stabilizer.

[0045] Item 32: In certain embodiments of item 31, the stabilizer is selected from the group consisting of a PEGylated lipid, and a surfactant.

[0046] Item 33: In certain embodiments of item 28, the payload is a nucleic acid, a protein, or a ribonucleoprotein.

[0047] Item 34: In certain embodiments of item 33, the payload is a nucleic acid.

[0048] Item 35: In certain embodiments of item 34, the nucleic acid is a ribonucleic acid.

[0049] **Item 36:** In certain embodiments of item 35, the ribonucleic acid is selected from antisense RNA, mRNA, o-RNA, miRNA, siRNA, or any combination thereof.

[0050] **Item 37:** In certain embodiments of any one of items 28-36, further comprise a delivery-enhancing peptide.

[0051] **Item 38:** Certain embodiments provide a method of delivering a payload to a cell, comprises contacting the cell with the composition of any one of items 28-37.

[0052] **Item 39:** In certain embodiments of item 38, the cell is a mammalian cell.

[0053] **Item 40:** In certain embodiments of item 39, the contacting is performed *in vitro*, *ex-vivo,* or *in vivo*.

[0054] **Item 41:** Certain embodiments provide a method for administering a therapeutic agent to a patient in need thereof, the method comprising preparing or providing the composition of any one of items 28-37 and administering the composition to the patient.

[0055] **Item 42:** Certain embodiments provide a use of a composition according to any of items 28-37 in the manufacture of a medicament.

[0056] The skilled artisan will recognize that, although the molecules of the invention are shown here for convenience in their neutral (unprotonated) forms, these molecules will exist in a partially or fully protonated form in solutions of appropriate pH, and that the present invention encompasses the molecules in all their protonated, unprotonated, ionized and non-ionized forms without limitation, unless specifically indicated otherwise.

GENERAL DEFINITIONS

[0057] The following definitions are included for the purpose of understanding the present subject matter and for constructing the appended patent claims. The abbreviations used herein have their conventional meanings within the chemical and biological arts.

[0058] While various embodiments and aspects of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood

that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[0059] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0060] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. *See, e.g.,* Singleton et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0061] The term "about" when used in reference to numerical ranges, cutoffs, or specific values is used to indicate that the recited values may vary by up to as much as 25% from the listed value. As many of the numerical values used herein are experimentally determined, it should be understood by those skilled in the art that such determinations can, and often times will, vary among different experiments. The values used herein should not be considered unduly limiting by virtue of this inherent variation. The term "about" is used to encompass variations of ±25% or less, variations of ±20% or less, variations of 10% or less, variations of ±5% or less, variations of ±1% or less, variations of ±0.5% or less, or variations of ±0.1% or less from the specified value. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

[0062] In the descriptions herein and in the claims, phrases such as "at least one of" or "one or more of" may occur followed by a conjunctive list of elements or features. The term "and/or" may also occur in a list of two or more elements or features. Unless otherwise implicitly or

explicitly contradicted by the context in which it is used, such a phrase is intended to mean any of the listed elements or features individually or any of the recited elements or features in combination with any of the other recited elements or features. For example, the phrases "at least one of A and B;" "one or more of A and B;" and "A and/or B" are each intended to mean "A alone, B alone, or A and B together." A similar interpretation is also intended for lists including three or more items. For example, the phrases "at least one of A, B, and C;" "one or more of A, B, and C;" and "A, B, and/or C" are each intended to mean "A alone, B alone, C alone, A and B together, A and C together, B and C together, or A and B and C together." In addition, use of the term "based on," above and in the claims is intended to mean, "based at least in part on," such that an unrecited feature or element is also permissible.

[0063] It is understood that where a parameter range is provided, all integers within that range, and tenths thereof, are also provided by the invention. For example, "0.2-5 mg" is a disclosure of 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg etc. up to and including 5.0 mg.

[0064] Compounds are generally described herein using standard nomenclature. For a recited compound having asymmetric center(s), all of the stereoisomers of the compound and mixtures thereof are encompassed unless otherwise specified. Non-limiting examples of stereoisomers include enantiomers, diastereomers, and E or Z isomers. Where a recited compound exists in various tautomeric forms, the compound is intended to encompass all tautomeric forms. Certain compounds are described herein using general formulas that include variables (e.g., X, L_1, L_2, L_3, Y, *etc.*). Unless otherwise specified, each variable within such a formula is defined independently of any other variable, and any variable that occurs more than one time in a formula is defined independently at each occurrence. If moieties are described as being "independently" selected from a group, each moiety is selected independently from the other. Each moiety therefore can be identical to or different from the other moiety or moieties.

[0065] The number of carbon atoms in a hydrocarbyl moiety can be indicated by the prefix "C_x-C_y," where x is the minimum and y is the maximum number of carbon atoms in the moiety. Thus, for example, "C_1-C_6 alkyl" refers to an alkyl substituent containing from 1 to 6 carbon atoms. Illustrating further, C_3-C_6 cycloalkyl means a saturated hydrocarbyl ring containing from 3 to 6 carbon ring atoms. A prefix attached to a multiple-component substituent only applies to the first component that immediately follows the prefix. To illustrate, the term

"carbocyclylalkyl" contains two components: carbocyclyl and alkyl. Thus, for example, C_3-C_6 carbocyclyl C_1-C_6 alkyl refers to a C_3-C_6 carbocyclyl appended to the parent molecular moiety through a C_1-C_6 alkyl group.

[0066] Unless otherwise specified, when a linking element links two other elements in a depicted chemical structure, the leftmost-described component of the linking element is bound to the left element in the depicted structure, and the rightmost-described component of the linking element is bound to the right element in the depicted structure. To illustrate, if the chemical structure is -L_S-M-L_S"- and M is -N(R_B)S(O)-, then the chemical structure is -L_S-N(R_B)S(O)-L_S"-.

[0067] If a linking element in a depicted structure is a bond, then the element left to the linking element is joined directly to the element right to the linking element via a covalent bond. For example, if a chemical structure is depicted as -L_S-M-L_S' and M is selected as bond, then the chemical structure will be -L_S-L_S"-. If two or more adjacent linking elements in a depicted structure are bonds, then the element left to these linking elements is joined directly to the element right to these linking elements via a covalent bond. For instance, if a chemical structure is depicted as -L_S-M-L_S"-M'-L_S"-, and M and L_S' are selected as bonds, then the chemical structure will be -L_S-M'-L_S"-. Likewise, if a chemical structure is depicted as -L_S-M-L_S"-M'-L_S"-, and M, L_S' and M' are bonds, then the chemical structure will be -L_S-L_S". When a chemical formula is used to describe a moiety, the dash(es) indicates the portion of the moiety that has the free valence(s).

[0068] If a moiety is described as being "optionally substituted", the moiety may be either substituted or unsubstituted. If a moiety is described as being optionally substituted with up to a particular number of non-hydrogen radicals that moiety may be either unsubstituted, or substituted by up to that particular number of non-hydrogen radicals or by up to the maximum number of substitutable positions on the moiety, whichever is less. Thus, for example, if a moiety is described as a heterocycle optionally substituted with up to three non-hydrogen radicals, then any heterocycle with less than three substitutable positions will be optionally substituted by up to only as many non-hydrogen radicals as the heterocycle has substitutable positions. For example, tetrazolyl (which has only one substitutable position) will be optionally substituted with up to one non-hydrogen radical. Similarly, if an amino nitrogen is described as

being optionally substituted with up to two non-hydrogen radicals, then a primary amino nitrogen will be optionally substituted with up to two non-hydrogen radicals, whereas a secondary amino nitrogen will be optionally substituted with up to only one non-hydrogen radical.

[0069] Where a moiety is substituted with oxo or thioxo, it means that the moiety contains a carbon atom covalently bonded to at least two hydrogens (e.g., CH_2), and the two hydrogen radicals are substituted with oxo or thioxo to form C=O or C=S, respectively.

[0070] The term "alkenyl" means a straight or branched hydrocarbyl chain containing one or more double bonds. Each carbon-carbon double bond may have either E (cis) or Z (trans) geometry within the alkenyl moiety, relative to groups substituted on the double bond carbons. Examples of alkenyl radicals include, but are not limited to, ethenyl, E- and Z-propenyl, isopropenyl, E- and Z-butenyl, E- and Z-isobutenyl, E- and Z-pentenyl, E- and Z-hexenyl, E,E-, E,Z-, Z,E- and Z,Z-hexadienyl and the like.

[0071] The term "alkenylene" refers to a divalent unsaturated hydrocarbyl chain which may be linear or branched and which has at least one carbon-carbon double bond. Non-limiting examples of alkenylene groups include -C(H)=C(H)-, -C(H)=C(H)-CH_2-, -C(H)=C(H)-CH_2-CH_2-, -CH_2-C(H)=C(H)-CH_2-, -C(H)=C(H)-CH-(CH_3)-, and -CH_2-C(H)=C(H)-CH-(CH_2CH_3)-.

[0072] The term "alkyl" means a straight or branched saturated hydrocarbyl chain. Non-limiting examples of alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, pentyl, iso-amyl, and hexyl.

[0073] The term "alkylene" denotes a divalent saturated hydrocarbyl chain which may be linear or branched. Representative examples of alkylene include, but are not limited to, -CH_2-, -CH_2CH_2-, -$CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2$-, and -$CH_2CH(CH_3)CH_2$-.

[0074] The term "alkynyl" means a straight or branched hydrocarbyl chain containing one or more triple bonds. Non-limiting examples of alkynyl include ethynyl, 1-propynyl, 2-propynyl, 3-propynyl, decynyl, 1-butynyl, 2-butynyl, and 3-butynyl.

[0075] The term "alkynyl," alone or in combination with any other term, refers to a straight-chain or branched-chain hydrocarbon radical having one or more triple bonds containing the

specified number of carbon atoms, or where no number is specified, in one embodiment from 2 to about 10 carbon atoms. Examples of alkynyl radicals include, but are not limited to, ethynyl, propynyl, propargyl, butynyl, pentynyl and the like.

[0076] The term "alkoxy" refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Examples of suitable alkyl ether radicals include, but are not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy and the like.

[0077] The term "aryl," alone or in combination with any other term, refers to a carbocyclic aromatic radical (such as phenyl or naphthyl) containing the specified number of carbon atoms, in one embodiment from 6-15 carbon atoms (i.e. (C_{6-15})aryl), and in another embodiment from 6-10 carbon atoms (i.e. (C_{6-10})aryl), optionally substituted with one or more substituents selected from alkyl, alkoxy, (for example methoxy), nitro, halogen, (for example chloro), amino, carboxylate and hydroxy. Examples of aryl radicals include, but are not limited to phenyl, p-tolyl, 4-hydroxyphenyl, 1-naphthyl, 2-naphthyl, indenyl, indanyl, azulenyl, fluorenyl, anthracenyl and the like.

[0078] The term "aralkyl", alone or in combination, means an alkyl radical as defined above in which one hydrogen atom is phenyl, benzyl, 2-phenylethyl and the like.

[0079] The term "aralkoxycarbonyl", alone or in combination, means a radical of the formula -C(O)-O-aralkyl in which the term "aralkyl" has the significance given above. An example of an aralkoxycarbonyl radical is benzyloxycarbonyl.

[0080] The term "aryloxy", alone or in combination, means a radical of the formula aryl-O- in which the term "aryl" has the significance given above.

[0081] The term "alkynylene" refers to a divalent unsaturated hydrocarbon group which may be linear or branched and which has at least one carbon-carbon triple bonds. Representative alkynylene groups include, by way of example, -C≡C-, -C≡C-CH_2-, -C≡C-CH_2-CH_2-, -CH_2-C≡C-CH_2-, -C≡C-CH(CH_3)-, and -CH_2-C≡C-CH(CH_2CH_3)-.

[0082] The term "alkanoyl", alone or in combination, means an acyl radical derived from an alkanecarboxylic acid, examples of which include acetyl, propionyl, butyryl, valeryl, 4-methylvaleryl, and the like.

[0083] The term "aryloxyalkanoyl" means an acyl radical of the formula aryl-O-alkanoyl wherein aryl and alkanoyl have the significance given above.

[0084] The term "aralkanoyl" means an acyl radical derived from an aryl-substituted alkanecarboxylic acid such as phenylacetyl, 3-phenylpropionyl (hydrocinnamoyl), 4-phenylbutyryl, (2-naphthyl)acetyl, 4-chlorohydrocinnamoyl, 4-aminohydrocinnamoyl, 4-phenylbutyryl, (1-naphthyl)acetyl, 4-chlorohydrocinnamoyl, 4-aminohydrocinnamoyl, 4-methoxyhydrocinnamoyl, and the like.

[0085] The term "aroyl" means an acyl radical derived from an aromatic carboxylic acid. Examples of such radicals include aromatic carboxylic acids, an optionally substituted benzoic or naphthoic acid such as benzoyl, 4-chlorobenzoyl, 4-carboxybenzoyl, 4-benzyloxycarbonyl)benzoyl, 1-naphthoyl, 2-naphthoyl, 6-carboxy-2-naphthoyl, 6-(benzyloxycarbonyl)-2-naphthoyl, 3-benzyloxy-2-naphthoyl, 3-hydroxy-2-naphthoyl, 3-(benzyloxyformamido)-2-naphthoyl, and the like.

[0086] The term "aminocarbonyl" alone or in combination, means an amino-substituted carbonyl (carbamoyl) group derived from an amino-substituted carboxylic acid wherein the amino group can be a primary, secondary or tertiary amino group continuing substituents selected from hydrogen, alkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl radicals and the like.

[0087] The term "aminoalkanoyl" means an acyl radical derived from an amino substituted alkanecarboxylic acid wherein the amino group can be a primary, secondary or tertiary amino group containing substituents selected from the group consisting of hydrogen, cycloalkyl, cycloalkylalkyl radicals and the like, examples of which include N,N-dimethylaminoacetyl and N-benzylaminoacetyl.

[0088] The term "carbocycle" or "carbocyclic" or "carbocyclyl" refers to a saturated (e.g., "cycloalkyl"), partially saturated (e.g., "cycloalkenyl" or "cycloalkynyl") or completely unsaturated (e.g., "aryl") 3- to 8-membered carbon ring system containing zero heteroatom ring atom. "Ring atoms" or "ring members" are the atoms bound together to form the ring or rings. A carbocyclyl may be, without limitation, a single ring, two fused rings, or bridged or spiro rings. A substituted carbocyclyl may have either cis or trans geometry. Representative examples of carbocyclyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl,

cyclohexyl, cycloheptyl, cyclooctyl, cyclopentenyl, cyclopentadienyl, cyclohexadienyl, adamantyl, decahydro-naphthalenyl, octahydro-indenyl, cyclohexenyl, phenyl, naphthyl, indanyl, 1,2,3,4-tetrahydro-naphthyl, indenyl, isoindenyl, decalinyl, and norpinanyl. A carbocycle group can be attached to the parent molecular moiety through any substitutable carbon ring atom. Where a carbocycle group is a divalent moiety linking two other elements in a depicted chemical structure, the carbocycle group can be attached to the two other elements through any two substitutable ring atoms. Likewise, where a carbocycle group is a trivalent moiety linking three other elements in a depicted chemical structure, the carbocycle group can be attached to the three other elements through any three substitutable ring atoms, respectively. The carbocycle may be attached at any endocyclic carbon atom which results in a stable structure. Carbocycles in one embodiment have 5-7 carbons.

[0089] The term "cycloalkyl" refers to a saturated carbocyclyl group containing zero heteroatom ring member. Non-limiting examples of cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, decalinyl and norpinanyl.

[0090] The term "cycloalkyl", alone or in combination, means an alkyl radical which contains from about 3 to about 8 carbon atoms and is cyclic. Examples of such cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

[0091] The term "cycloalkylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkyl radical containing from about 3 to about 8, in one embodiment from about 3 to about 6, carbon atoms.

[0092] The term "cycloalkylcarbonyl" means an acyl group derived from a monocyclic or bridged cycloalkanecarboxylic acid such as cyclopropanecarbonyl, cyclohexanecarbonyl, adamantanecarbonyl, and the like, or from a benz-fused monocyclic cycloalkanecarboxylic acid which is optionally substituted by, for example, alkanoylamino, such as 1,2,3,4-tetrahydro-2-naphthoyl, 2-acetamido-1,2,3,4-tetrahydro-2-naphthoyl.

[0093] The term "cycloalkylalkoxycarbonyl" means an acyl group derived from a cycloalkylalkoxycarboxylic acid of the formula cycloalkylalkyl-O-COOH wherein cycloalkylalkyl has the significance given above.

[0094] The term "carbocyclylalkyl" refers to a carbocyclyl group appended to the parent molecular moiety through an alkylene group. For instance, C_3-C_6carbocyclylC_1-C_6alkyl refers to a C_3-C_6carbocyclyl group appended to the parent molecular moiety through C_1-C_6alkylene.

[0095] The term "cycloalkenyl" refers to a non-aromatic, partially unsaturated carbocyclyl moiety having zero heteroatom ring member. Representative examples of cycloalkenyl groups include, but are not limited to, cyclobutenyl, cyclopentenyl, cyclohexenyl, and octahydronaphthalenyl.

[0096] The prefix "halo" indicates that the substituent to which the prefix is attached is substituted with one or more independently selected halogen radicals. For example, "C_1-C_6haloalkyl" means a C_1-C_6alkyl substituent wherein one or more hydrogen atoms are replaced with independently selected halogen radicals. Non-limiting examples of C_1-C_6haloalkyl include chloromethyl, 1-bromoethyl, fluoromethyl, difluoromethyl, trifluoromethyl, and 1,1,1-trifluoroethyl. It should be recognized that if a substituent is substituted by more than one halogen radical, those halogen radicals may be identical or different (unless otherwise stated).

[0097] The term "heterocycle" or "heterocyclo" or "heterocyclyl" refers to a saturated (e.g., "heterocycloalkyl"), partially unsaturated (e.g., "heterocycloalkenyl" or "heterocycloalkynyl") or completely unsaturated (e.g., "heteroaryl") ring system where at least one of the ring atoms is a heteroatom (i.e., nitrogen, oxygen or sulfur), with the remaining ring atoms being independently selected from the group consisting of carbon, nitrogen, oxygen and sulfur. A heterocycle may be, without limitation, a single ring, two fused rings, or bridged or spiro rings. A heterocycle group can be linked to the parent molecular moiety via any substitutable carbon or nitrogen atom(s) in the group. Where a heterocycle group is a divalent moiety that links two other elements in a depicted chemical structure, the heterocycle group can be attached to the two other elements through any two substitutable ring atoms. Likewise, where a heterocycle group is a trivalent moiety that links three other elements in a depicted chemical structure, the heterocycle group can be attached to the three other elements through any three substitutable ring atoms, respectively.

[0098] In the instant compounds, “Het” indicates a heterocycle containing 4-12 carbon atom, where at least one nitrogen atom is present in the ring(s). A heterocyclyl may be, without limitation, a monocycle which contains a single ring. Non-limiting examples of monocycles

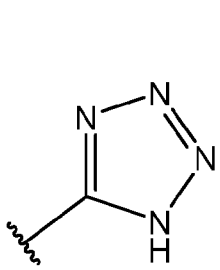
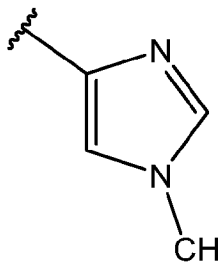
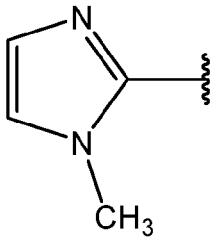
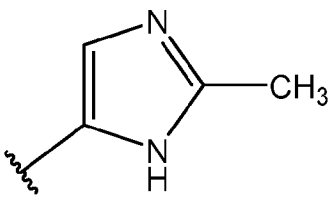
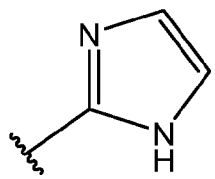
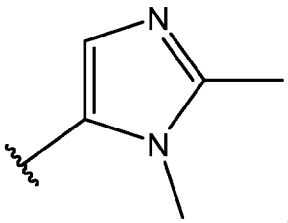
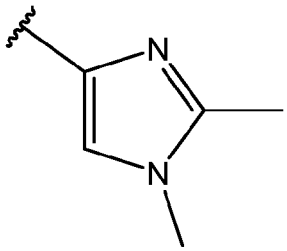
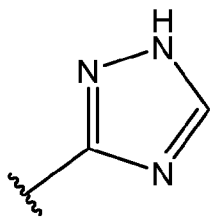
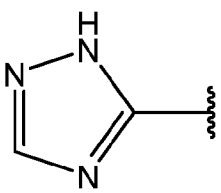
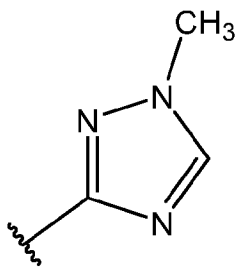
include furanyl, dihydrofuranyl, tetrahydrofuranyl, pyrrolyl, isopyrrolyl, pyrrolinyl, pyrrolidinyl, imidazolyl, isoimidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, triazolyl, tetrazolyl, dithiolyl, oxathiolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, thiazolinyl, isothiazolinyl, thiazolidinyl, isothiazolidinyl, thiodiazolyl, oxathiazolyl, oxadiazolyl (including 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl (also known as "azoximyl"), 1,2,5-oxadiazolyl (also known as "furazanyl"), and 1,3,4-oxadiazolyl), oxatriazolyl (including 1,2,3,4-oxatriazolyl and 1,2,3,5-oxatriazolyl), dioxazolyl (including 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, and 1,3,4-dioxazolyl), pyridinyl, piperidinyl, diazinyl (including pyridazinyl (also known as "1,2-diazinyl"), pyrimidinyl (also known as "1,3-diazinyl"), and pyrazinyl (also known as "1,4-diazinyl")), piperazinyl, triazinyl (including s-triazinyl (also known as "1,3,5-triazinyl"), as-triazinyl (also known 1,2,4-triazinyl), and v-triazinyl (also known as "1,2,3-triazinyl), oxazinyl (including 1,2,3-oxazinyl, 1,3,2-oxazinyl, 1,3,6-oxazinyl (also known as "pentoxazolyl"), 1,2,6-oxazinyl, and 1,4-oxazinyl), isoxazinyl (including o-isoxazinyl and p-isoxazinyl), oxazolidinyl, isoxazolidinyl, oxathiazinyl (including 1,2,5-oxathiazinyl or 1,2,6-oxathiazinyl), oxadiazinyl (including 1,4,2-oxadiazinyl and 1,3,5,2-oxadiazinyl), morpholinyl, azepinyl, and diazepinyl.

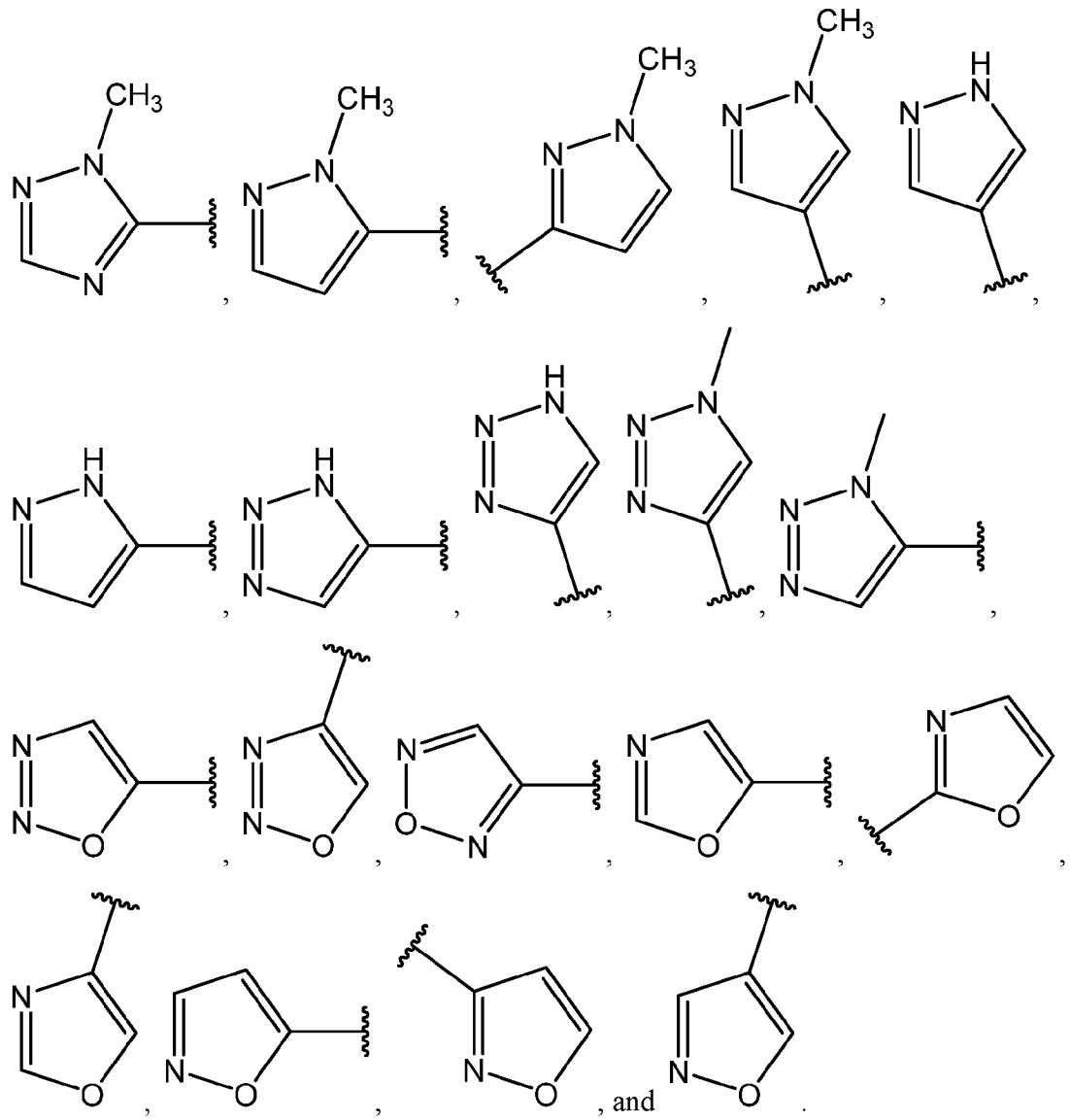
[0099] A heterocyclyl may also be, without limitation, a bicycle containing two fused rings, such as, for example, naphthyridinyl (including [1,8]naphthyridinyl, and [1,6]naphthyridinyl), thiazolpyrimidinyl, thienopyrimidinyl, pyrimidopyrimidinyl, pyridopyrimidinyl, pyrazolopyrimidinyl, indolizinyl, pyrindinyl, pyranopyrrolyl, 4H-quinolizinyl, purinyl, pyridopyridinyl (including pyrido[3,4-b]-pyridinyl, pyrido[3,2-b]-pyridinyl, and pyrido[4,3-b]-pyridinyl), pyridopyrimidine, and pteridinyl. Other non-limiting examples of fused-ring heterocycles include benzo-fused heterocyclyls, such as indolyl, isoindolyl, indoleninyl (also known as "pseudoindolyl"), isoindazolyl (also known as "benzpyrazolyl" or indazolyl), benzazinyl (including quinolinyl (also known as "1-benzazinyl") and isoquinolinyl (also known as "2-benzazinyl")), benzimidazolyl, phthalazinyl, quinoxalinyl, benzodiazinyl (including cinnolinyl (also known as "1,2-benzodiazinyl") and quinazolinyl (also known as "1,3-benzodiazinyl")), benzothiazolyl, 4,5,6,7-tetrahydrobenzo[d]thiazolyl, benzothiadiazolyl, benzimidazolyl, benzotriazolyl, benzoxazinyl (including 1,3,2-benzoxazinyl, 1,4,2-benzoxazinyl, 2,3,1-benzoxazinyl, and 3,1,4-benzoxazinyl), benzisoxazinyl (including 1,2-benzisoxazinyl and 1,4-benzisoxazinyl), and tetrahydroisoquinolinyl.

[0100] A heterocyclyl may also be, without limitation, a spiro ring system, such as, for example, 1,4-dioxa-8-azaspiro[4.5]decanyl. A heterocyclyl may comprise one or more sulfur atoms as ring members; and in some cases, the sulfur atom(s) is oxidized to SO or SO_2. The nitrogen heteroatom(s) in a heterocyclyl may or may not be quaternized, and may or may not be oxidized to N-oxide. In addition, the nitrogen heteroatom(s) may or may not be N-protected.

[0101] A heterocycle or carbocycle may be further substituted. Unless specified, the term "substituted" refers to substitution by independent replacement of one, two, or three or more of the hydrogen atoms with substituents including, but not limited to, -F, -Cl, -Br, -I, hydroxy, protected hydroxy, $-NO_2$, $-N_3$, -CN, $-NH_2$, protected amino, oxo, thioxo, -NH-C_2-C_8-alkenyl, -NH-C_2-C_8-alkynyl, -NH-C_3-C_{12}-cycloalkyl, -NH-aryl, -NH-heteroaryl, -NH-heterocycloalkyl, -dialkylamino, -diarylamino, -diheteroarylamino, -O-C_1-C_{12}-alkyl, -O-C_2-C_8-alkenyl, alkynyl, -O-C_3-C_{12}-cycloalkyl, -O-aryl, -O-heteroaryl, -O-heterocycloalkyl, -C(O)-C_1-C_{12}-alkyl, -C(O)-C_2-C_8-alkenyl, -C(O)-C_2-C_8-alkynyl, -C(O)-C_3-C_{12}-cycloalkyl, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-heterocycloalkyl, $-CONH_2$, -CONH-C_1-C_{12}-alkyl, -CONH-C_2-C_8-alkenyl, -CONH-C_2-C_8-alkynyl, -CONH-C_3-C_{12}-cycloalkyl, -CONH-aryl, -CONH-heteroaryl, -CONH-heterocycloalkyl, -OCO_2-C_1-C_{12}-alkyl, -OCO_2-C_2-C_8-alkenyl, -OCO_2-C_2-C_8-alkynyl, -OCO_2-C_3-C_{12}-cycloalkyl, -OCO_2-aryl, -OCO_2-heteroaryl, -OCO_2-heterocycloalkyl, $-OCONH_2$, -OCONH-C_1-C_{12}-alkyl, -OCONH-C_2-C_8-alkenyl, -OCONH-C_2-C_8-alkynyl, -OCONH-C_3-C_{12}-cycloalkyl, -OCONH-aryl, -OCONH-heteroaryl, -OCONH-heterocycloalkyl, -NHC(O)-C_1-C_{12}-alkyl, -NHC(O)-C_2-C_8-alkenyl, -NHC(O)-C_2-C_8-alkynyl, -NHC(O)-C_3-C_{12}-cycloalkyl, -NHC(O)-aryl, -NHC(O)-heteroaryl, -NHC(O)-heterocycloalkyl, -$NHCO_2$-C_1-C_{12}-alkyl, -$NHCO_2$-C_2-C_8-alkenyl, -$NHCO_2$-C_2-C_8-alkynyl, -$NHCO_2$-C_3-C_{12}-cycloalkyl, -$NHCO_2$-aryl, -$NHCO_2$-heteroaryl, -$NHCO_2$-heterocycloalkyl, $-NHC(O)NH_2$, -NHC(O)NH-C_1-C_{12}-alkyl, -NHC(O)NH-C_2-C_8-alkenyl, -NHC(O)NH-C_2-C_8-alkynyl, -NHC(O)NH-C_3-C_{12}-cycloalkyl, -NHC(O)NH-aryl, -NHC(O)NH-heteroaryl, -NHC(O)NH-heterocycloalkyl, $-NHC(S)NH_2$, -NHC(S)NH-C_1-C_{12}-alkyl, -NHC(S)NH-C_2-C_8-alkenyl, -NHC(S)NH-C_2-C_8-alkynyl, -NHC(S)NH-C_3-C_{12}-cycloalkyl, -NHC(S)NH-aryl, -NHC(S)NH-heteroaryl, -NHC(S)NH-heterocycloalkyl, $-NHC(NH)NH_2$, -NHC(NH)NH-C_1-C_{12}-alkyl, -NHC(NH)NH-C_2-C_8-alkenyl, NHC(NH)NH-C_2-C_8-alkynyl, -NHC(NH)NH-C_3-C_{12}-cycloalkyl, -NHC(NH)NH-aryl, -NHC(NH)NH-heteroaryl, -NHC(NH)NH-heterocycloalkyl, -NHC(NH)-C_1-C_{12}-alkyl, -NHC(NH)-C_2-C_8-alkenyl, -NHC(NH)-C_2-C_8-alkynyl, -NHC(NH)-C_3-C_{12}-cycloalkyl, -NHC(NH)-aryl, -NHC(NH)-

heteroaryl, -NHC(NH)-heterocycloalkyl, -C(NH)NH-C_1-C_{12}-alkyl, -C(NH)NH-C_2-C_8-alkenyl, -C(NH)NH-C_2-C_8-alkynyl, -C(NH)NH-C_3-C_{12}-cycloalkyl, -C(NH)NH-aryl, -C(NH)NH-heteroaryl, -C(NH)NH-heterocycloalkyl, -S(O)-C_1-C_{12}-alkyl, -S(O)-C_2-C_8-alkenyl, -S(O)-C_2-C_8-alkynyl, -S(O)-C_3-C_{12}-cycloalkyl, -S(O)-aryl, -S(O)-heteroaryl, -S(O)-heterocycloalkyl, -SO_2NH_2, -SO_2NH-C_1-C_{12}-alkyl, -SO_2NH-C_2-C_8-alkenyl, -SO_2NH-C_2-C_8-alkynyl, -SO_2NH-C_3-C_{12}-cycloalkyl, -SO_2NH-aryl, -SO_2NH-heteroaryl, -SO_2NH-heterocycloalkyl, -$NHSO_2$-C_1-C_{12}-alkyl, -$NHSO_2$-C_2-C_8-alkenyl, -$NHSO_2$-C_2-C_8-alkynyl, -$NHSO_2$-C_3-C_{12}-cycloalkyl, -$NHSO_2$-aryl, -$NHSO_2$-heteroaryl, -$NHSO_2$-heterocycloalkyl, -CH_2NH_2, -$CH_2SO_2CH_3$, -aryl, -arylalkyl, -heteroaryl, -heteroarylalkyl, -heterocycloalkyl, -C_3-C_{12}-cycloalkyl, polyalkoxyalkyl, polyalkoxy, -methoxymethoxy, -methoxyethoxy, -SH, -S-C_1-C_{12}-alkyl, -S-C_2-C_8-alkenyl, -S-C_2-C_8-alkynyl, -S-C_3-C_{12}-cycloalkyl, -S-aryl, -heteroaryl, -S-heterocycloalkyl, or methylthiomethyl. It is understood that the aryls, heteroaryls, alkyls, and the like can be further substituted.

[0102] In some embodiments, the heterocycle is a 5-7 membered monocyclic basic heterocycle selected from the group consisting of  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  .



[0103] The term "N-protecting group" or "N-protected" refers to those groups capable of protecting an amino group against undesirable reactions. Commonly used N-protecting groups are described in Greene and Wuts, *Protecting Groups in Chemical Synthesis* (3rd ed., John Wiley & Sons, NY (1999)). Non-limiting examples of N-protecting groups include acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, or 4-nitrobenzoyl; sulfonyl groups such as benzenesulfonyl or p-toluenesulfonyl; sulfenyl groups such as phenylsulfenyl (phenyl-S-) or triphenylmethylsulfenyl (trityl-S-); sulfinyl groups such as p-methylphenylsulfinyl (p-methylphenyl-S(O)-) or t-butylsulfinyl (t-Bu-S(O)-); carbamate forming

groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxy carbonyl, dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxy carbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloro-ethoxy-carbonyl, phenoxy carbonyl, 4-nitro-phenoxy carbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, or phenylthiocarbonyl; alkyl groups such as benzyl, p-methoxybenzyl, triphenylmethyl, or benzyloxymethyl; p-methoxyphenyl; and silyl groups such as trimethylsilyl. Preferred N-protecting groups include formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, benzyl, t-butyloxycarbonyl (Boc) and benzyloxycarbonyl (Cbz).

[0104] The term "halogen" means fluorine, chlorine, bromine or iodine.

[0105] The term “ionizable lipid” refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH 4 and a neutral charge at other pHs such as physiological pH 7.

[0106] The term "lysis agent" or “endosomal release agent” as used herein refers to a molecule, compound, protein or peptide which is capable of breaking down an endosomal membrane and freeing the DNA transporter into the cytoplasm of the cell. This term includes but is not limited to viruses, synthetic compounds, lytic peptides, or derivatives thereof. The term "lytic peptide" refers to a chemical grouping which penetrates a membrane such that the structural organization and integrity of the membrane is lost. As a result of the presence of the lysis agent, the membrane undergoes lysis, fusion or both. Examples of lysis agents/endosomal release agents include choroquine, polyamines and polyamidoamines. Suitable agents are described in, for example, Pei and Buyanova, *Bioconjugate Chem,* **30**:273-283 (2009) and Juliano, *Nucleic Acid Therapeutics*, 28:166-177 (2018).

[0107] The term “polycationic nucleic acid binding moiety” as used herein refers to a moiety containing multiple positive charges at physiological pH that allow the moiety to bind a

negatively charged nucleic acid. A polycationic nucleic acid binding moiety may be linked to, for example, a cell surface ligand, a fusion agent, and/or a nuclear localization peptide. The linkage may be covalent. Suitable polycationic nucleic acid binding moieties include polyamines such as PEI, spermine, spermidine, carboxyspermine and polybasic peptides containing, for example, multiple lysine, ornithine, histidine, or arginine residues.

[0108] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may in embodiments be conjugated to a moiety that does not consist of amino acids. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a chimeric protein encoding two or more separate protein sequences that are recombinantly expressed or chemically synthesized as a single moiety.

[0109] The term "stabilizing agent" refers to a compound that mitigates the degradation of the lipid nanoparticles or a subpopulation of the lipid nanoparticles.

[0110] The term "structural lipid" refers to sterols and lipids containing sterol like moieties.

[0111] The term "surface ligand" or "cell surface ligand" refers to a chemical compound or structure which will bind to a surface receptor of a cell. The term "cell surface receptor" as used herein refers to a specific chemical grouping on the surface of a cell to which the ligand can attach. Cell surface receptors can be specific for a particular cell, i.e., found predominantly in one cell rather than in another type of cell (e.g., LDL and asialoglycoprotein receptors are specific for hepatocytes). The receptor facilitates the internalization of the ligand and attached molecules. A cell surface receptor includes but is not limited to a folate receptor, biotin receptor, lipoic acid receptor, low-density lipoprotein receptor, asialoglycoprotein receptor, insulin-like growth factor type II/cation-independent mannose-6-phosphate receptor, calcitonin gene-related peptide receptor, insulin-like growth factor I receptor, nicotinic acetylcholine receptor, hepatocyte growth factor receptor, endothelin receptor, bile acid receptor, bone morphogenetic protein receptor, cartilage induction factor receptor or glycosylphosphatidylinositol (GPI)-

anchored proteins (*e.g.*, β-adrenergic receptor, T-cell activating protein, Thy-1 protein, GPI-anchored 5' nucleotidase). These are nonlimiting examples.

[0112] A "receptor" is a molecule to which a ligand binds specifically and with relatively high affinity. A receptor is usually a protein or a glycoprotein, but may also be a glycolipid, a lipidpolysaccharide, a glycosaminoglycan or a glycocalyx. For purposes of this disclosure, epitopes to which an antibody or its fragments binds is construed as a receptor since the antigen:antibody complex undergoes endocytosis. Furthermore, surface ligand includes anything which is capable of entering the cell through cytosis (e.g. endocytosis, potocytosis, pinocytosis).

[0113] As used herein, the term "ligand" refers to a chemical compound or structure which will bind to a receptor. This includes but is not limited to ligands such as asialoorosomucoid, asialoglycoprotein, lipoic acid, biotin, apolipoprotein E sequence, insulin-like growth factor II, calcitonin gene-related peptide, thymopoietin, hepatocyte growth factor, endothelin-1, atrial natriuretic factor, RGD-containing cell adhesion peptides and the like. The ligand may also be a plant virus movement protein or peptide derived from such a protein. Suitable peptides and proteins are described, for example, in US Patent No. 10,538,784, the contents of which are hereby incorporated by reference in their entirety.

[0114] One skilled in the art will readily recognize that a ligand chosen will depend on which receptor is being bound. Since different types of cells have different receptors, this provides one method of targeting nucleic acid to specific cell types, depending on which cell surface ligand is used. Thus, use of a cell surface ligand may depend on the targeted cell type.

SYNTHESIS OF THE LIPIDS

[0115] The synthetic processes of the disclosure can tolerate a wide variety of functional groups, therefore various substituted starting materials can be used. The processes generally provide the desired final compound at or near the end of the overall process, although it may be desirable in certain instances to further convert the compound to a pharmaceutically acceptable salt thereof.

[0116] Compounds of the present disclosure can be prepared in a variety of ways using commercially available starting materials, compounds known in the literature, or from readily prepared intermediates, by employing standard synthetic methods and procedures either known to those skilled in the art, or which will be apparent to the skilled artisan in light of the teachings herein. Standard synthetic methods and procedures for the preparation of organic molecules and functional group transformations and manipulations can be obtained from the relevant scientific literature or from standard textbooks in the field. Although not limited to any one or several sources, classic texts such as Smith, M. B., March, J., March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition, John Wiley & Sons: New York, 2001; Greene, T. W., Wuts, P. G. M., Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons: New York, 1999; R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), incorporated by reference herein, are useful and recognized reference textbooks of organic synthesis known to those in the art. The following descriptions of synthetic methods are designed to illustrate, but not to limit, general procedures for the preparation of compounds of the present disclosure.

[0116] The following example schemes are provided for the guidance of the reader, and represent preferred methods for making the compounds exemplified herein. These methods are not limiting, and it will be apparent that other routes may be employed to prepare these compounds. Such methods specifically include solid phase based chemistries, including combinatorial chemistry. The skilled artisan is thoroughly equipped to prepare these compounds by those methods given the literature and this disclosure. The compound numberings used in the synthetic schemes depicted below are meant for those specific schemes only, and should not be construed as or confused with same numberings in other sections of the application.

[0117] Trademarks used herein are examples only and reflect illustrative materials used at the time of the invention. The skilled artisan will recognize that variations in lot, manufacturing processes, and the like, are expected. Hence the examples, and the trademarks used in them are non-limiting, and they are not intended to be limiting, but are merely an illustration of how a skilled artisan may choose to perform one or more of the embodiments of the invention.

[0118] The following abbreviations have the indicated meanings:

DCM	= dichloromethane
DIEA	= N,N-Diisopropylethylamine
DIPEA	= N,N-Diisopropylethylamine
DMF	= N,N-dimethylformamide
DMP	= Dess Martin Periodinane
DNs	= dinitrosulfonyl
ESBL	= extended-spectrum β-lactamase
EtOAc	= ethyl acetate
EA	= ethyl acetate
FCC	= Flash Column Chromatography
HATU	= 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
MeCN	= acetonitrile
NMR	= nuclear magnetic resonance
PE	= Petroleum Ether
Prep	= preparatory
Py	= pyridine
RT	= room temperature
Sat.	= saturated aqueous
TBDMSCl	= *tert*-butyldimethylsilyl chloride
TBS	= *tert*-butyldimethylsilyl
TFA	= trifluoroacetic acid
THF	= tetrahydrofuran
TLC	= thin layer chromatography

[0117] The compounds of this disclosure having any of the formulae described herein may be prepared according to the procedures illustrated in Schemes 1, 2, 3, and 4 below, from commercially available starting materials or starting materials which can be prepared using

literature procedures. The variables in the schemes (e.g., R_1, R_2, and R_3 etc. are as defined herein). One of ordinary skill in the art will note that, during the reaction sequences and synthetic schemes described herein, the order of certain steps may be changed, such as the introduction and removal of protecting groups.

[0118] One of ordinary skill in the art will recognize that certain groups may require protection from the reaction conditions via the use of protecting groups. Protecting groups may also be used to differentiate similar functional groups in molecules. A list of protecting groups and how to introduce and remove these groups can be found in Greene, T. W., Wuts, P. G. M., Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons: New York, 1999.

[0119] Preferred protecting groups include, but are not limited to:

For a hydroxyl moiety: TBS, benzyl, THP, Ac;

For carboxylic acids: benzyl ester, methyl ester, ethyl ester, allyl ester;

For amines: Fmoc, Cbz, BOC, DMB, Ac, Bn, Tr, Ts, trifluoroacetyl, phthalimide, benzylideneamine;

For diols: Ac (x2) TBS (x2), or when taken together acetonides;

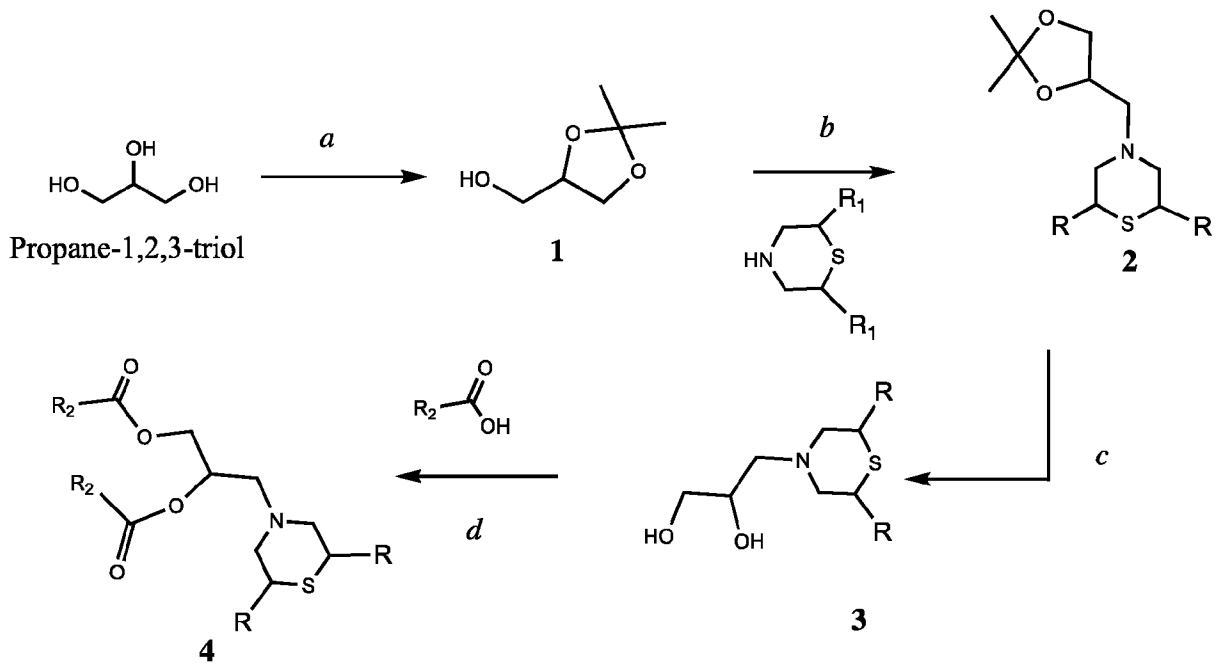
For thiols: Ac;

For benzimidazoles: SEM, benzyl, PMB, DMB;

For aldehydes: di-alkyl acetals such as dimethoxy acetal or diethyl acetyl.

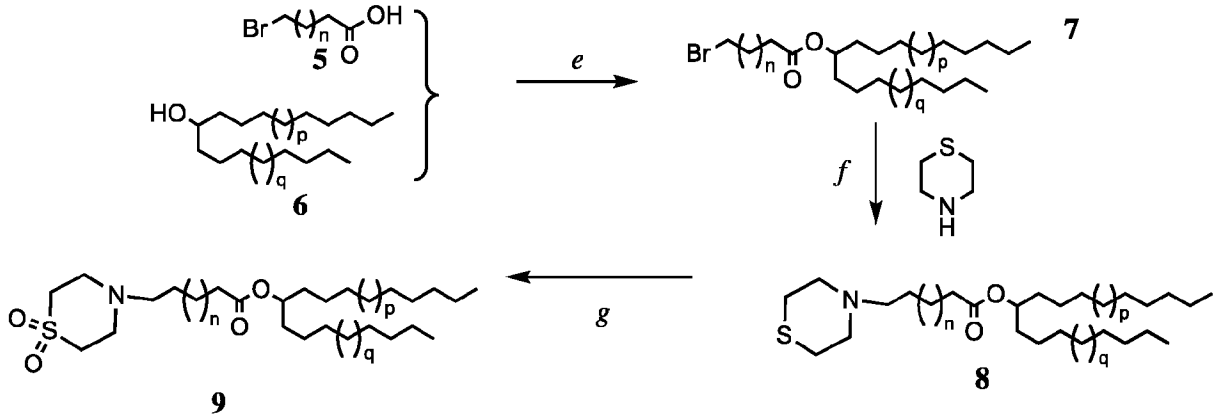
[0120] In the reaction schemes described herein, multiple stereoisomers may be produced. When no particular stereoisomer is indicated, it is understood to mean all possible stereoisomers that could be produced from the reaction. A person of ordinary skill in the art will recognize that the reactions can be optimized to give one isomer preferentially, or new schemes may be devised to produce a single isomer. If mixtures are produced, techniques such as preparative thin layer chromatography, preparative HPLC, preparative chiral HPLC, or preparative SFC may be used to separate the isomers.

[0121] Symmetric and asymmetric cationic lipids of general structure (I) may be synthesized using methods that are well known in the art, as shown, for example in Schemes 1, 2, 3, and 4.



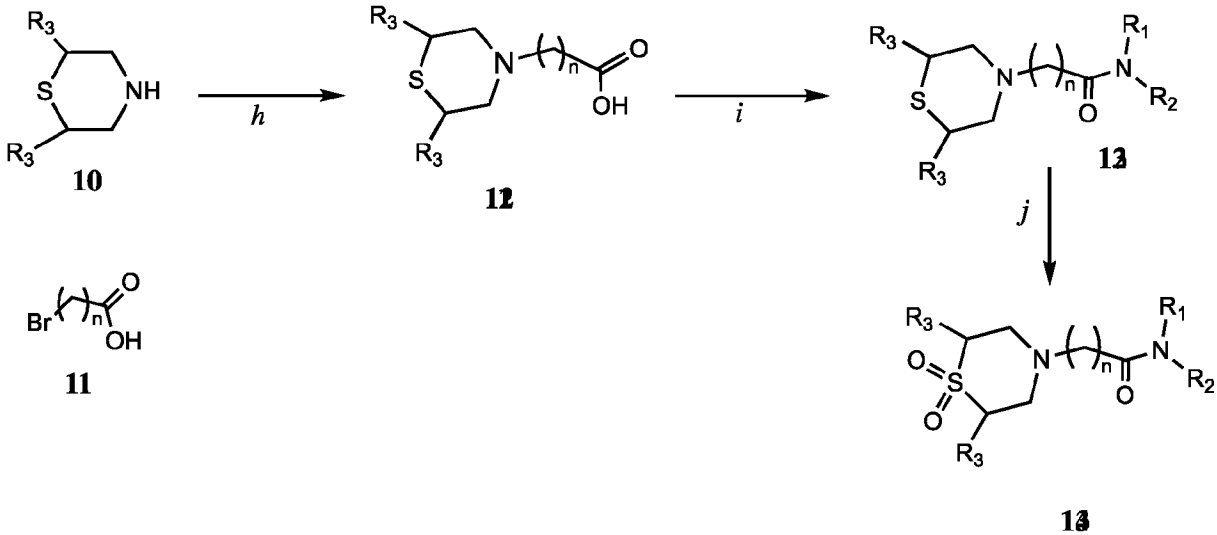
Scheme 1

[0122] As illustrated in Scheme 1 above, the claimed compounds may be produced by (*a*) reacting propane-1,2,3 triol with acetone in a solvent with a strong acid, typically THF in the presence of p-tolulene sulfonic acid, to form the acetal **1**. The free hydroxyl group may be converted (*b*) to **2** by a variety of methods, such as Mitsunobu coupling in which compound **1** is solubilized in THF or similar solvent with a thio morpholine analog in the presence of triphenyl phosphine. Typically, an azodicarboxylate such as diethyl azodicarboxylate (DEAD) is slowly added dropwise resulting in the formation of **2**. Deprotection of the hydroxyls may be accomplished by removing the acetal group with trifluoracetic in THF/H_2O (*c*) to form **3**. The hydroxyl groups may be subsequently esterified (*d*) to form **4** in the presence of a carbodiimide and a nucleophilic catalyst such as 4-Dimethylaminopyridine (DMAP).



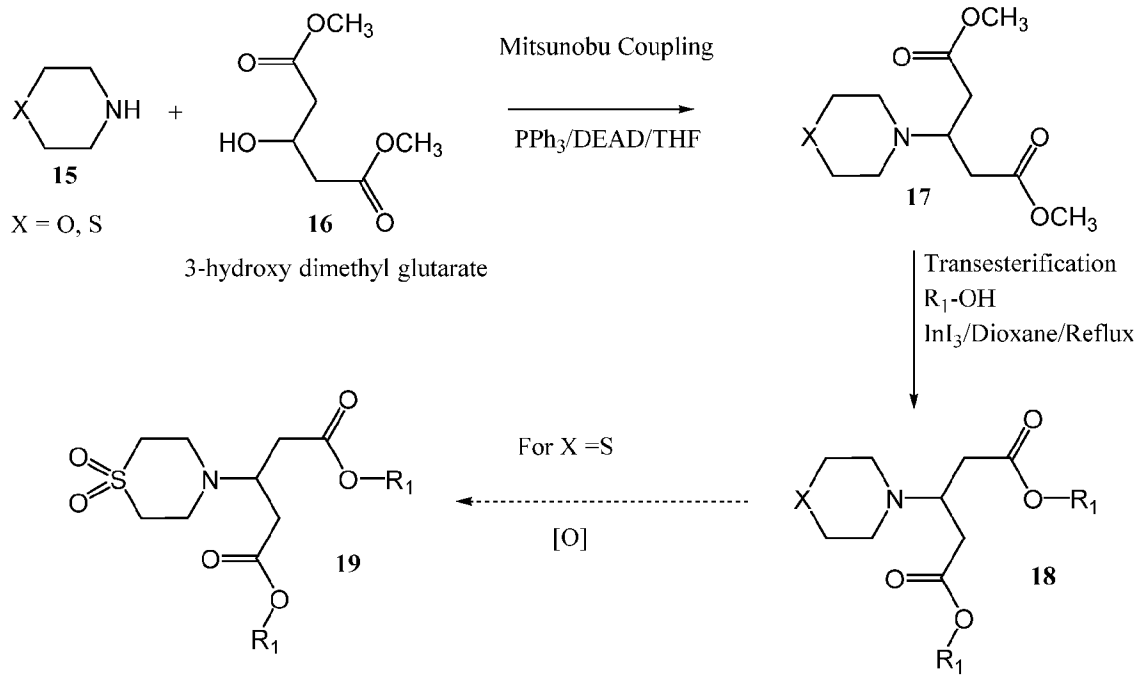
Scheme 2

[0123] Scheme 2 presents an alternative route to compounds of the invention. As illustrated a bromo alkyl acid **5** may react with an alcohol **6** to afford ester **7**. This step may take place in an organic solvent such as DMF in the presence of a carbodiimide, 4-dimethylaminopyridine and N,N-diisopropylethylamine. Compound **7** may react with thio morpholine when heated in DMF in the presence of N-diisopropylethylamine to produce **8**. Alternatively, **8** may be produced by incubating 7 and thiol morpholine in acetonitrile, NaI and Na_2CO_3. The thiol group may be oxidized with m-chloroperoxybenzoic or $K^+H_2SO_5^-$ in a methanol/water solution.



Scheme 3

[0124] Scheme 3 shows a third route of synthesis. The thio morpholine compound **10** may be heated with compound **11** in DMF in the presence of N-diisopropylethylamine to form compound **12**. Reaction of the acid moiety with a secondary amine results in the formation of the amid **13**. The thiol group may be further oxidized to form compound **14**.



Scheme 4

[0125] Scheme 4 shows a fourth route of synthesis. The (thio)morpholine compound **15** may be coupled to ester **16** using Mitsunobu coupling conditions to form homoligated compound **17**. Transesterification of the diester leads to diester **18**. In some instances, diester 18 may be oxidized to form compound **19**.

[0126] A person of ordinary skill in the art will recognize that in the above schemes the order of certain steps may be interchangeable.

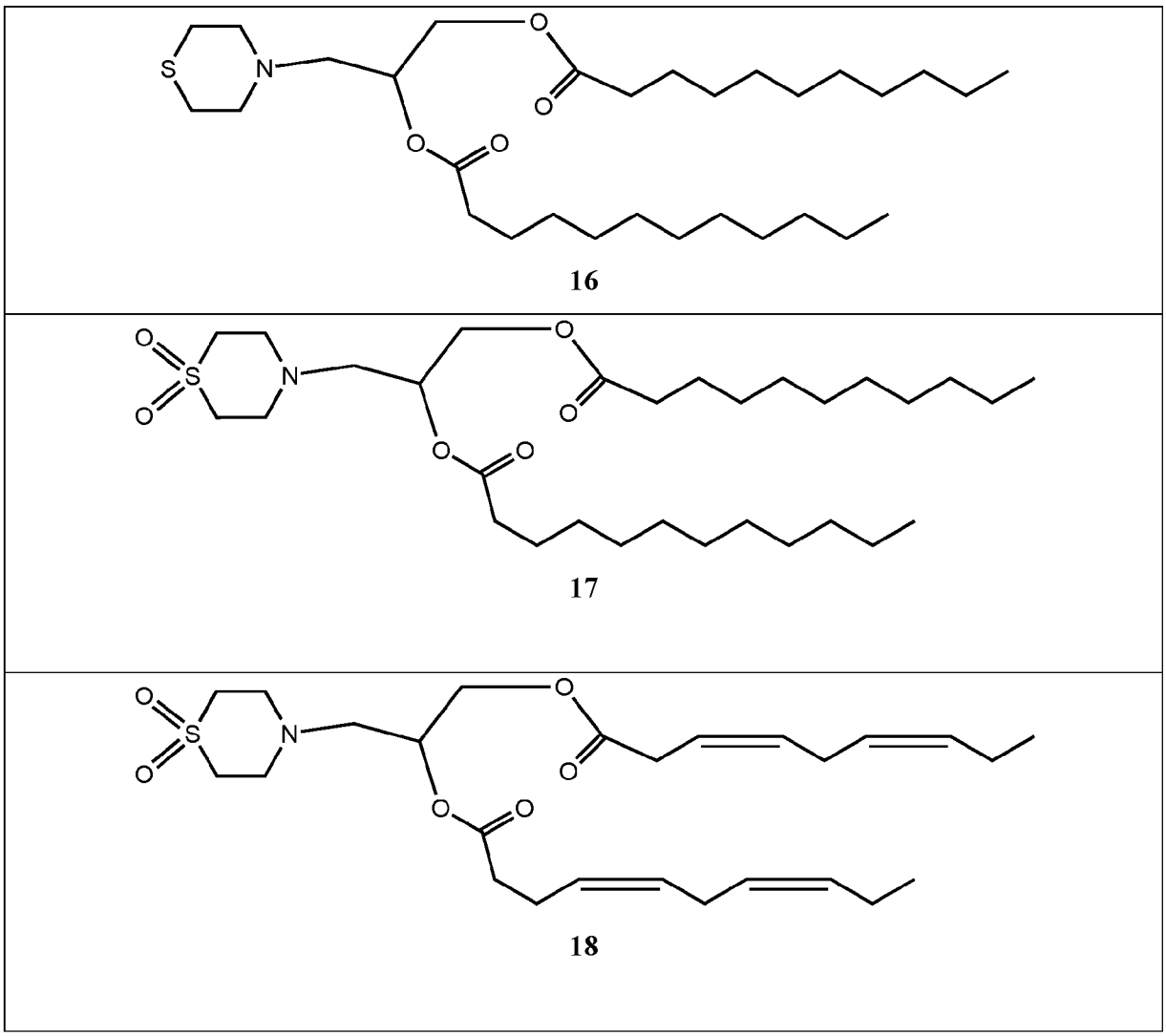
[0127] In certain aspects, the disclosure also includes methods of synthesizing a compound of any of Formulae (I), (I-A), (I-B), (I-C), (I-D), (I-E), (I-F), (I-G), (I-H), (I-I, (I-J), or (I-K) and intermediate(s) for synthesizing the compound.

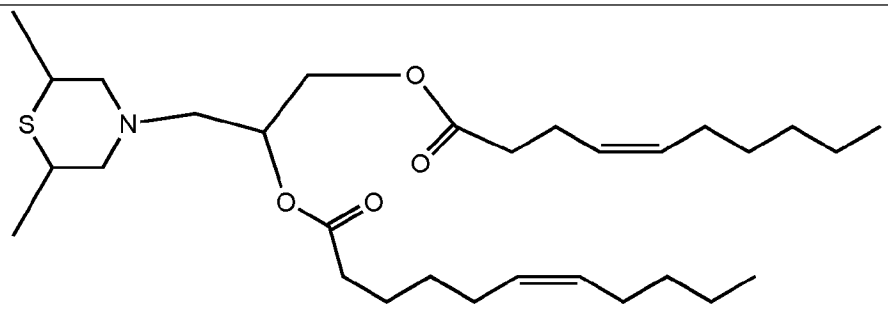
[0128] Other methods of preparing compounds of formula (I) will be apparent to the skilled artisan.

COMPOUNDS

[0129] Specific examples of compounds of structure (I) include compounds having the structure in Table I below.

Table I





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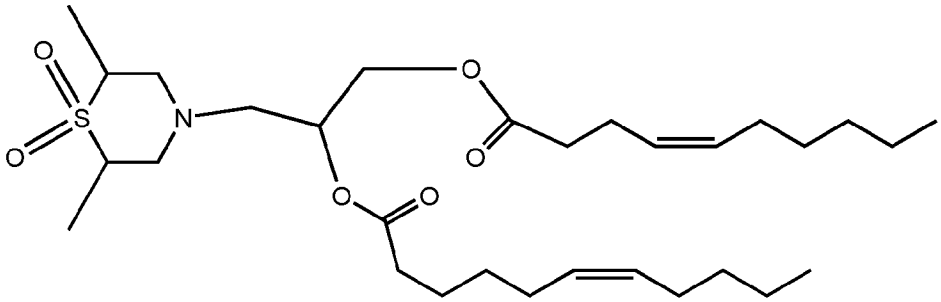

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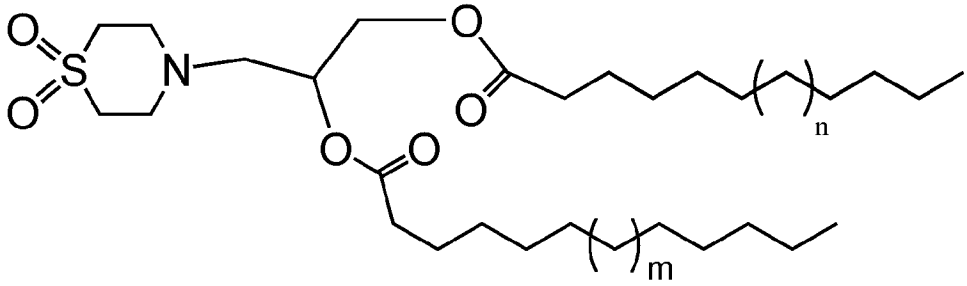

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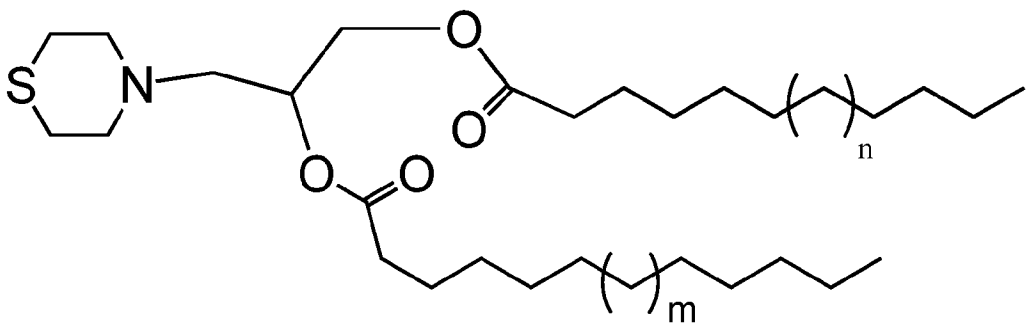

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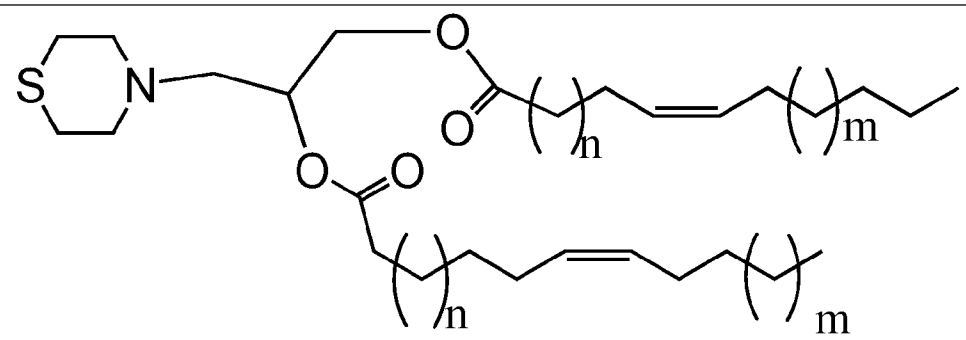

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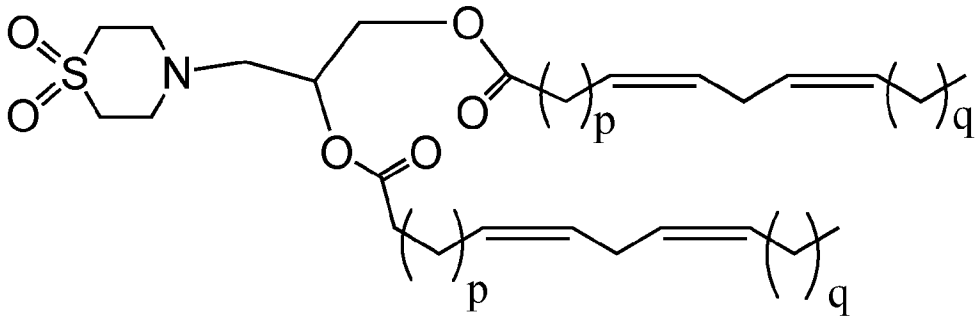

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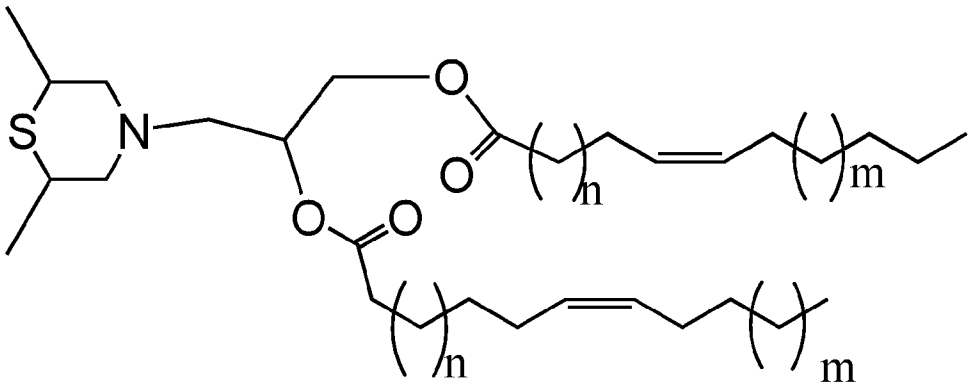

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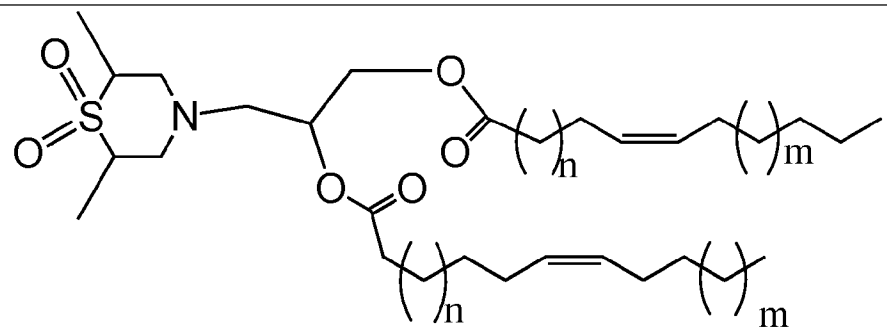

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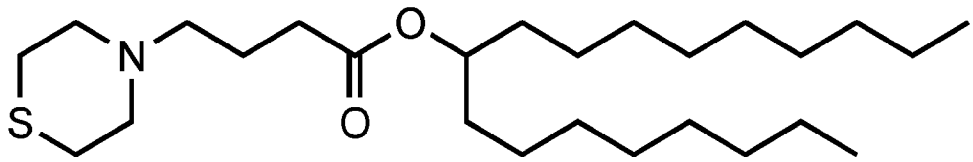

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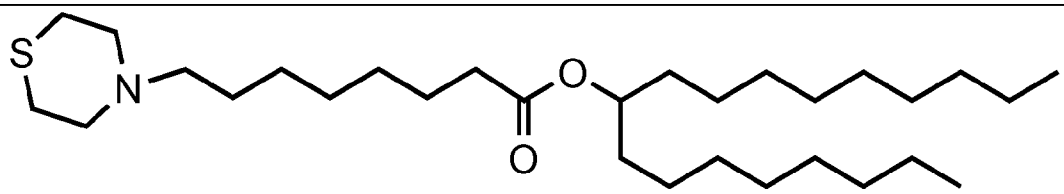

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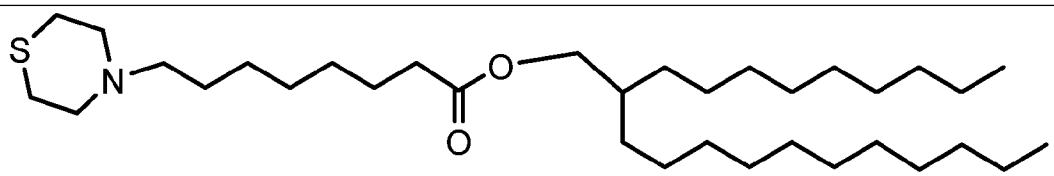

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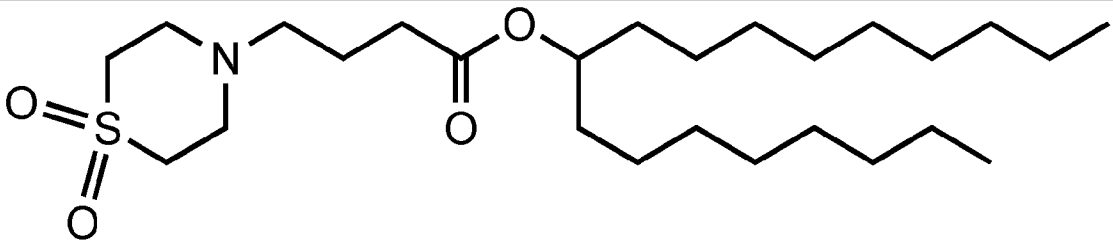

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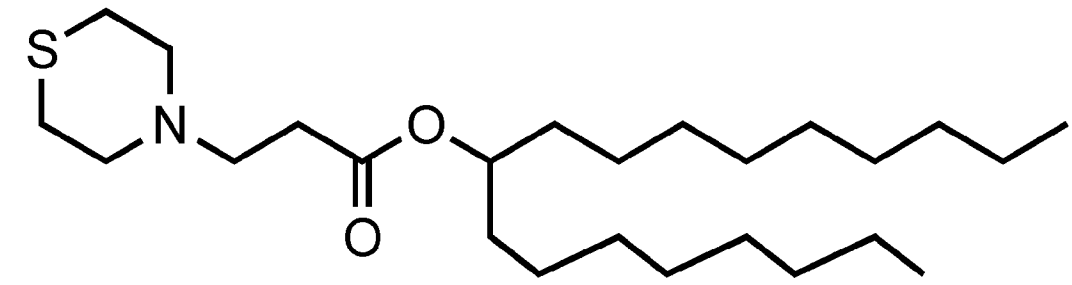

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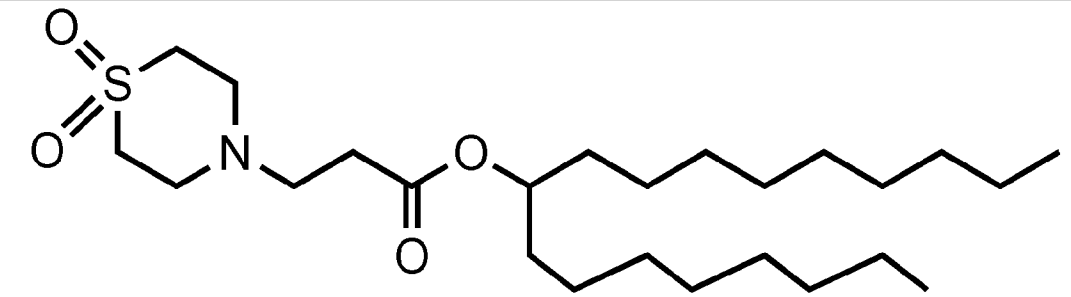

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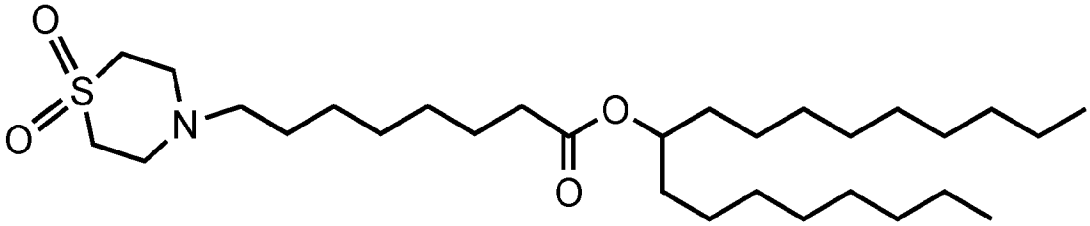

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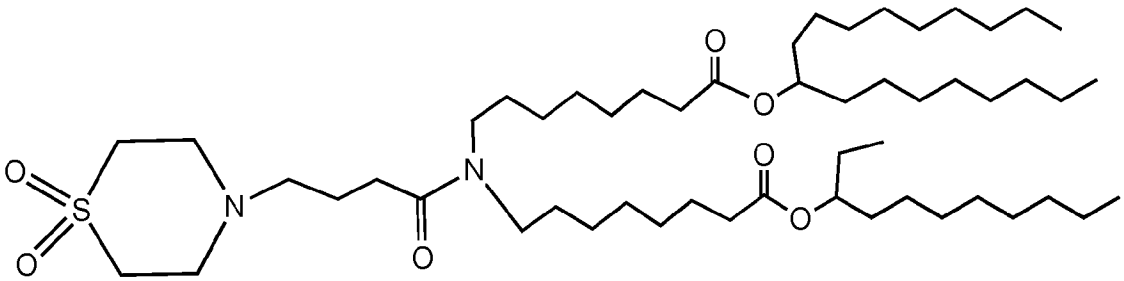

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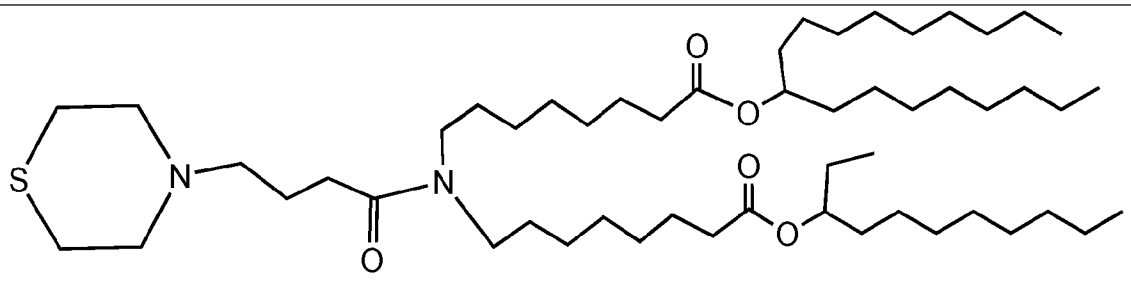

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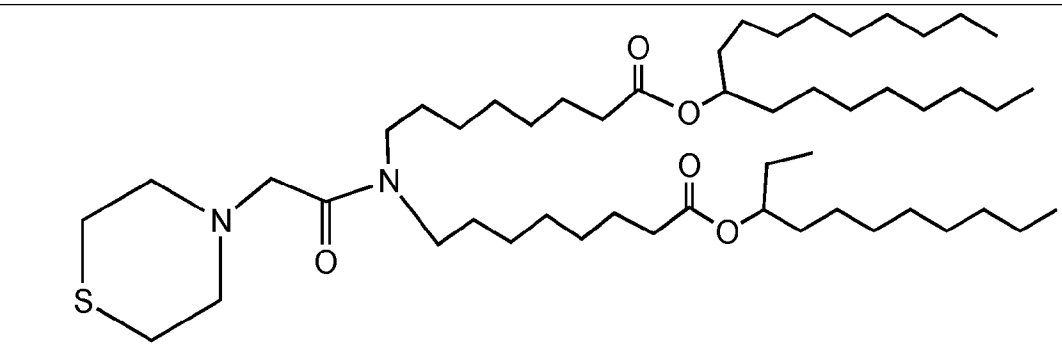

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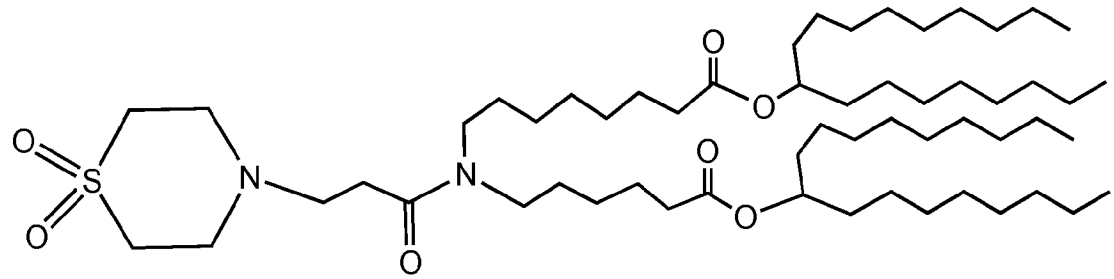

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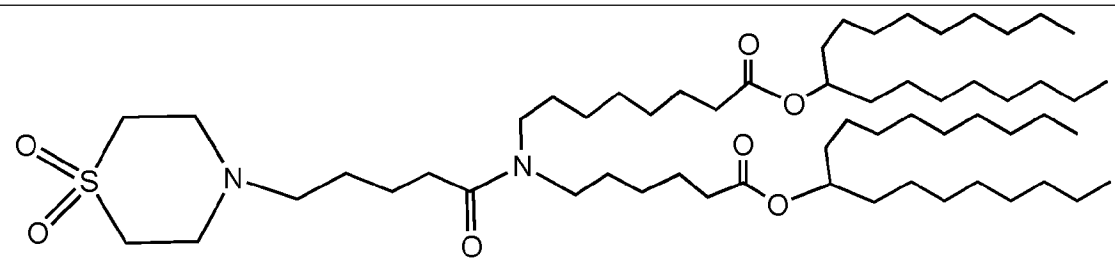

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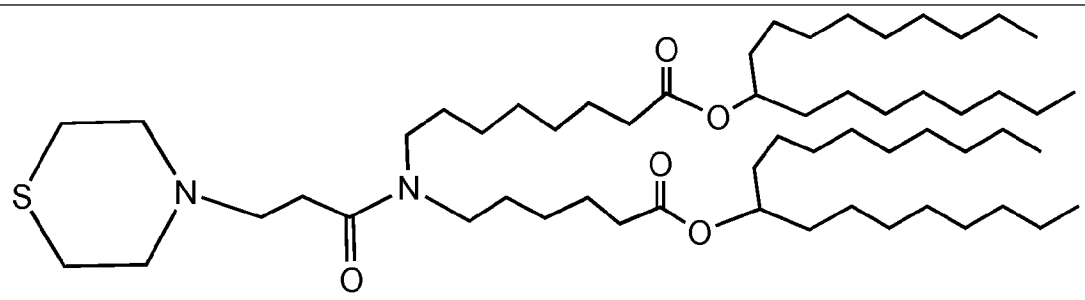

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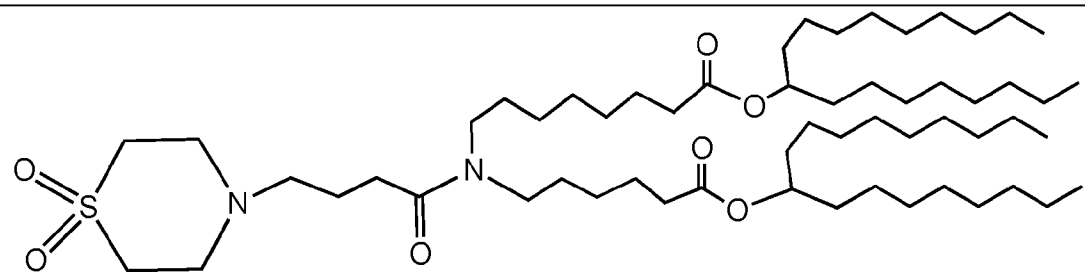

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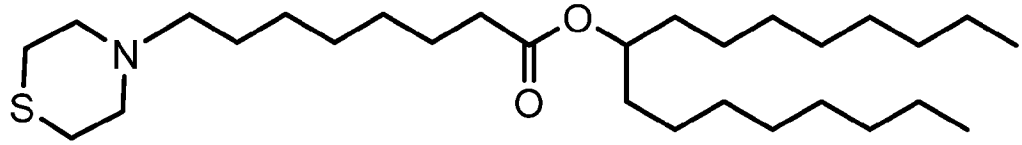

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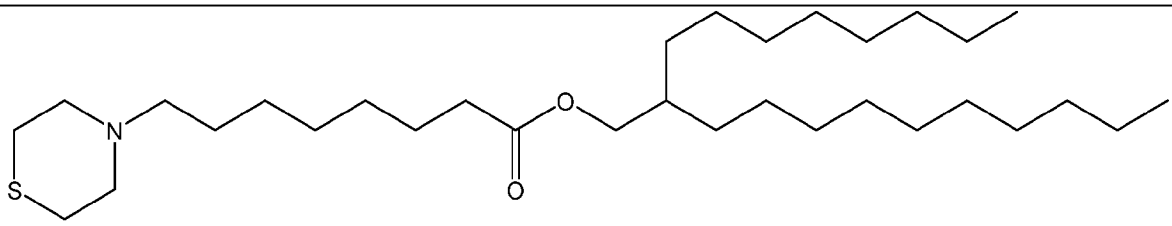

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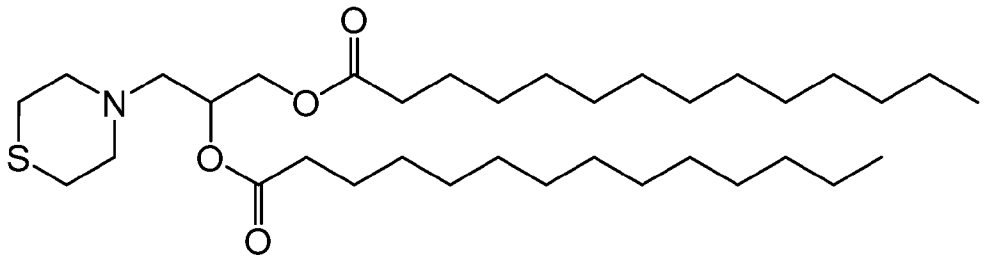

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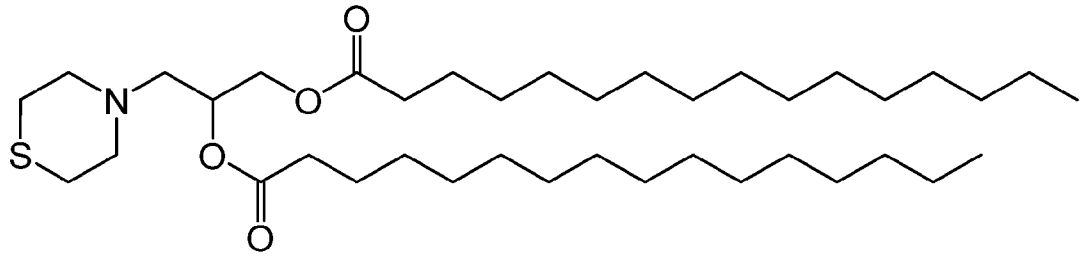

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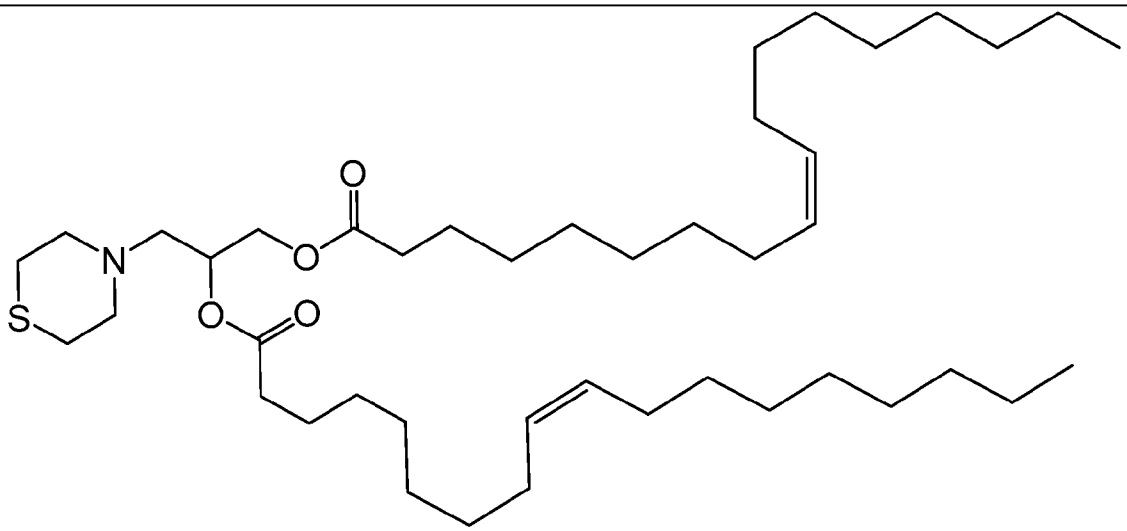

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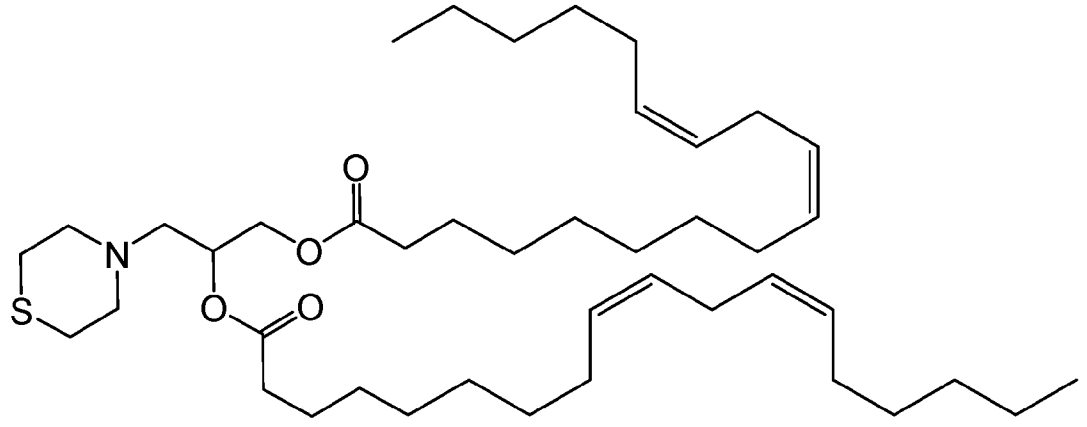

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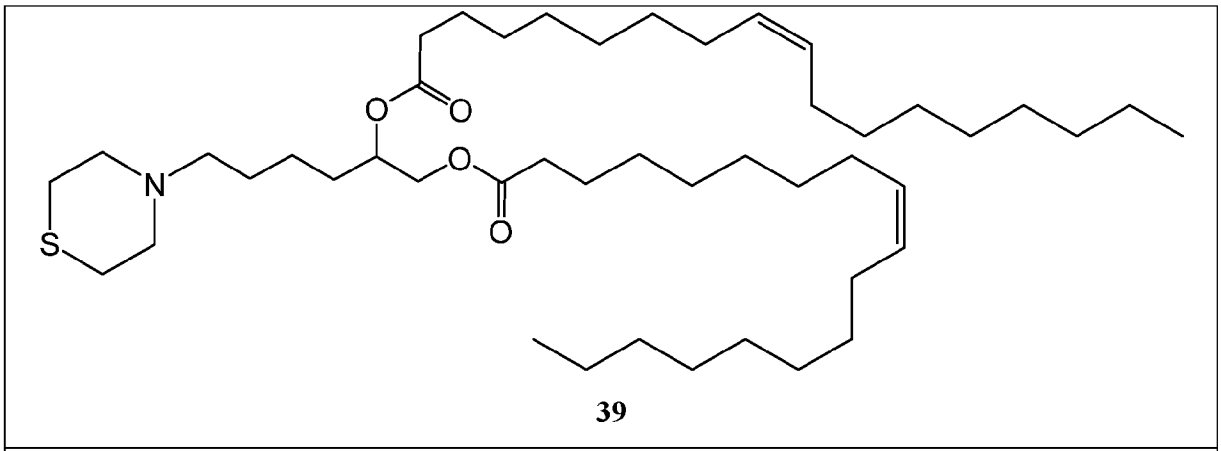

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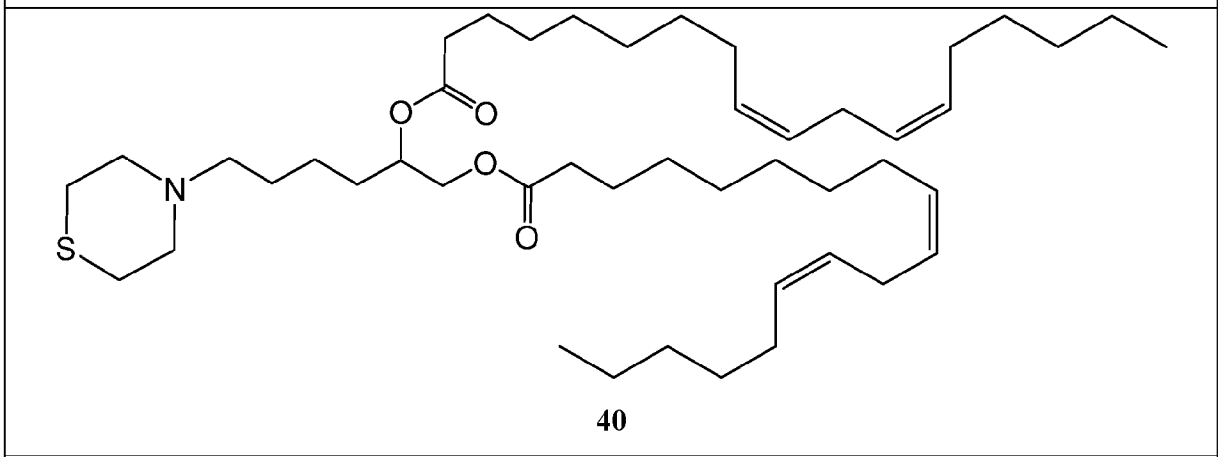

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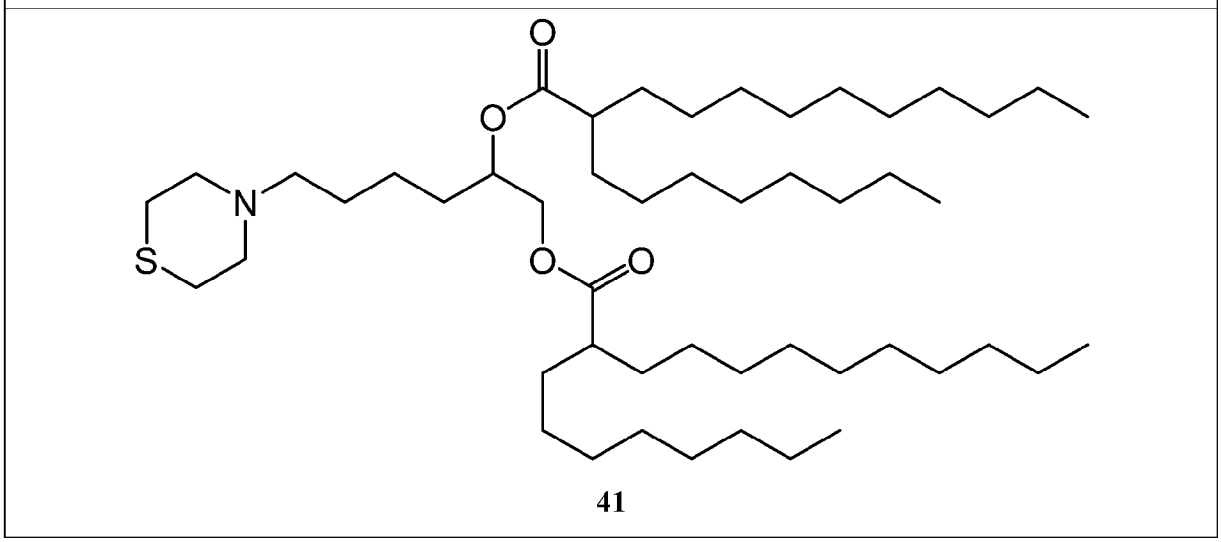

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COMPOSITIONS

[0130] The compositions provided herein encompass complexes in the form of lipid nanoparticles, liposomes (e.g., lipid vesicles) and lipoplexes. As used herein, the term

"liposome" encompasses any compartment enclosed by a lipid bilayer. The term liposome includes unilamellar vesicles which are comprised of a single lipid bilayer and generally have a diameter in the range of about 20 to about 400 nm. Liposomes can also be multilamellar having a diameter in the range of approximately 1 μm to approximately 10 μm. Multilamellar liposomes may consist of several (anywhere from two to hundreds) unilamellar vesicles forming one inside the other in diminishing size, creating a multilamellar structure of concentric phospholipid spheres separated by layers of water. Alternatively, multilamellar liposomes may consist of many smaller nonconcentric spheres of lipid inside a large liposome. In embodiments, liposomes include multilamellar vesicles (MLV), large unilamellar vesicles (LUV), and small unilamellar vesicles (SUV). In some embodiments, the compositions include liposomes which contain any suitable ionizable lipid and neutral lipids, along with the peptide as provided herein.

[0131] Another aspect of the present application relates to a composition including one or more compounds described herein, and one or more of a structural lipid, an ionizable lipid, and a stabilizing agent; and optionally, a payload.

[0132] In some embodiments, the compositions include a compound described herein; one or more structural lipids; one or more stabilizing agents; and optionally, a payload.

[0133] In some embodiments, the compositions include a compound described herein; one or more structural lipids; one or more stabilizing agents; one or more transfection enhancing agents; and optionally, a payload.

[0134] In some embodiments, the compositions include 10 to 80 mol% of one or more of a compound described herein, excluding any payload, if present.

Payload

[0135] An aspect of the present application relates to a composition including one or more compounds described herein, and a payload.

[0136] According to the present disclosure, compositions as described herein can further comprise one or more bioactive molecules to be delivered to a cell (i.e., a payload). For example, bioactive molecules can include, e.g., nucleic acids, peptides, active pharmaceutical agents, nutrients, small molecules, or the like. Preferably, the payloads in the embodiments provided herein are biomolecules, e.g., either nucleic acids (RNA, DNA, etc., as described in

more detail herein), peptides, or a combination thereof. In some embodiments, the payload can be a therapeutic and/or prophylactic agent. Alternatively, the payload can be used for cosmetic, or nutraceutical applications. The therapeutic and/or prophylactic agents are sometimes referred to as a "therapeutic payload" or "payload" in the present disclosure. In some embodiments, the payload can be administered in vivo or in vitro using the compositions provided herein as a delivery vehicle.

[0137] In some embodiments, the payload is a nucleic acid. In some embodiments, the compositions include a compound described herein with a charge N and a nucleic acid molecule with a charge P, where the combination of the compound and the nucleic acid contacting the cell comprises an N/P ratio from about 1 to 20.

[0138] In some embodiments, the nucleic acid is an RNA. In some embodiments, the RNA is mRNA, siRNA, shRNA, self-replicating RNA (srRNA), an o-RNA, self-amplifying RNA, stRNA, trRNA, crRNA, sgRNA, RNAi molecule, an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), or any combination thereof. In some embodiments, the RNA is an mRNA. The compositions can include two or more different mRNAs.

[0139] In some embodiments, the RNA encodes an immunogen. In some embodiments, the RNA encodes a cancer antigen

[0140] In some embodiments, the nucleic acid is a DNA.

[0141] In some embodiments, the payload includes one or more peptides, and optionally a nucleic acid. In some embodiments, the peptide is covalently linked to a nucleic acid.

Efficiency of encapsulation

[0142] The efficiency of encapsulation of a payload (e.g., macromolecule) describes the amount of payload that is encapsulated or otherwise associated with a lipid composition after preparation, relative to the initial amount provided. In some embodiments, the encapsulation efficiency is desirably high (e.g., close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of payload in a solution containing the lipid complex composition before and after breaking up the lipid complex composition with one or more organic solvents or detergents. By way of example, for nucleic acid payloads, fluorescence may

be used to measure the amount of free payload (e.g., RNA or DNA) in a solution. In some embodiments for the lipid compositions described herein, the encapsulation efficiency of a payload may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In certain embodiments, the encapsulation efficiency may be at least 90%.

[0143] By way of example, for RNA payloads, e.g., mRNA, the encapsulation efficiency (EE%) can be measured using a fluorescence plate-based assay employing the RIBOGREEN™ reagent. This assay measures the quantity of mRNA in samples with intact lipid complexes (e.g, lipid nanoparticles, or "LNPs") to determine the quantity of unencapsulated RNA as well as in LNP samples disrupted by triton X-100 to measure the total RNA. The % of encapsulation efficiency can be calculated as the difference between the total RNA and the unencapsulated RNA divided by the total RNA.

[0144] Advantageously, in addition to the lipids of formula (I), the lipid complexes provided herein include one or more co-lipids, most advantageously neutral co-lipids, although the skilled artisan will recognize that other lipids, including cationic/ionizable lipids, may be used. Some formulations, however, may include just the lipids of formula (I), in combination with a nucleic acid.

Lipid nanoparticles (LNPs)

[0145] In some embodiments, the compositions include lipid nanoparticles (LNPs). LNP compositions are typically sized on the order of micrometers or small and may include a lipid bilayer. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 1 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 900 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 800 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 700 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 600 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20

nm to about 500 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 400 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 300 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 200 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 100 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 20 nm to about 50 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 900 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 800 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 700 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 600 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 500 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 400 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 300 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 200 μm. In some embodiments, the lipid nanoparticle composition comprises a lipid formulation, wherein the size is from about 100 nm to about 150 μm.

Nucleic Acid

[0146] In some embodiments, the present lipid compositions include one or more nucleic acid molecules (e.g., DNA or RNA molecules) as the payload.

[0147] In some embodiments, the nucleic acid of the lipid complex composition is an RNA molecule. For example, the RNA payloads can be mRNA, siRNA, shRNA, miRNA, self-replicating RNA (srRNA), self-amplifying RNA (saRNA), stRNA, sgRNA, or combinations thereof. In some embodiments, the payload includes more than one mRNA molecule (e.g., at least 2 mRNA molecules, at least 3 mRNA molecules, at least four mRNA molecules, or at least

5 mRNA molecules). In some embodiments, the an RNA payload includes at least one sgRNA. In other embodiments, the nucleic acid of the lipid complex composition molecule includes an sgRNA molecule and an mRNA molecule.

[0148] In some embodiments, the lipid composition includes a gene editing reagent (or a gene editing composition), and the gene editing reagent includes a gene editing protein, an RNA or DNA (e.g., encoding a gene editing protein), a donor nucleic acid, a ribonucleoprotein (RNP), or any combination thereof. In various examples, the gene editing protein includes a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a Cas protein, a MegaTal, a Cre recombinase, a Hin Recombinase, or a Flp recombinase. In some embodiments, the RNA molecule includes sgRNA, a crRNA, and/or a tracrRNA. Accordingly, in some embodiments, the lipid complex composition includes an RNP and an sgRNA, and optionally a donor nucleic acid. In some embodiments, the RNP can include a Cas protein and a sgRNA, a crRNA or a tracrRNA, and optionally a donor nucleic acid.

[0149] In some embodiments, the payload can include a gene editing protein covalently or non-covalently bound to a donor nucleic acid (e.g., a donor DNA or a donor RNA).

[0150] In other embodiments, the payload includes a nucleic acid that encodes a gene editing protein (e.g., a DNA or an RNA encoding a ZFN, TALEN, Cas protein, Cre recombinase, etc). Accordingly, in some embodiments, the lipid complex composition includes an RNA encoding a gene editing protein and an sgRNA. In some embodiments, the lipid complex composition can include an RNA encoding a Cas protein and a sgRNA, a crRNA or a tracrRNA, and optionally a donor nucleic acid. In some embodiments, the payload includes a nucleic acid that encodes a therapeutic protein, such as an antibody, growth factor, cytokine, enzyme, or the like.

[0151] In some embodiments, the nucleic acid of the lipid complex compositions is a single-stranded molecule. In some embodiments, the nucleic acid of the lipid complex is double stranded. In some embodiments, the lipid may include donor DNA. In still other embodiments, the payload may be a plasmid DNA or linear DNA. In still other embodiments, the payload may be doggiebone DNA, or "dbDNA." In some embodiments, the payload can be an oligonucleotide, such as an antisense oligonucleotide.

[0152] In certain embodiments, the gene editing composition induces single-strand or double-strand breaks in DNA within the cells. In some embodiments the gene editing reagent (or gene editing composition) further comprises a repair template polynucleotide. In various embodiments, the repair template comprises (a) a first flanking region comprising nucleotides in a sequence complementary to about 40 to about 90 base pairs on one side of the single or double strand break and a second flanking region comprising nucleotides in a sequence complementary to about 40 to about 90 base pairs on the other side of the single or double strand break; or (b) a first flanking region comprising nucleotides in a sequence complementary to at least about 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, or 90 base pairs on one side of the single or double strand break and a second flanking region comprising nucleotides in a sequence complementary to at least about 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, or 90 base pairs on the other side of the single or double strand break. Non-limiting descriptions relating to gene editing (including repair templates) using the CRISPR-Cas system are discussed in Ran et al. (2013) Nat Protoc. 2013 Nov; 8(11): 2281-2308, the entire content of which is incorporated herein by reference. Embodiments involving repair templates are not limited to those comprising the CRISPR-Cas system.

[0153] Non-limiting examples of Cas proteins include Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csxl2), CaslO, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, CsxlO, Csxl6, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof. These enzymes are known; for example, the amino acid sequence of S. pyogenes Cas9 protein may be found in the SwissProt database under accession number Q99ZW2 and in the NCBI database as under accession number Q99ZW2.1. UniProt database accession numbers A0A0G4DEU5 and CDJ55032 provide another example of a Cas9 protein amino acid sequence. Another non-limiting example is a Streptococcus thermophilus Cas9 protein, the amino acid sequence of which may be found in the UniProt database under accession number Q03JI6.1. In some embodiments, the unmodified CRISPR enzyme has DNA cleavage activity, such as Cas9. In certain embodiments the CRISPR enzyme is Cas9, and may be Cas9 from S. pyogenes or S. pneumoniae. In various embodiments, the CRISPR enzyme directs cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. In some

embodiments, the CRISPR enzyme directs cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence. In some embodiments, a vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution in the RuvC I catalytic domain of Cas9 from S. pyogenes converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, H840A, N854A, and N863A. In aspects of the invention, nickases may be used for genome editing via homologous recombination.

[0154] In certain embodiments, a Cas9 nickase may be used in combination with guide sequence(s), e.g., two guide sequences, which target respectively sense and antisense strands of the DNA target. This combination allows both strands to be nicked and used to induce NHEJ.

[0155] In some embodiments, the nucleic acid of the lipid composition encodes for an immunogen. In some examples the nucleic acid of the lipid composition encodes for a hemagglutinin (HA) protein or fragment thereof. In some examples the nucleic acid of the lipid composition encodes for ovalbumin or fragment thereof. In examples, the lipid composition induces an immune response in a subject to the nucleic acid-encoded protein of the lipid composition.

[0156] In the instance of delivery of a nucleic acid, the amount of nucleic acid (e.g., DNA, mRNA, self-amplifying RNA, dbDNA or the like) in lipid nanoparticle formulation may depend on the size, sequence, and other characteristics of the nucleic acid. The amount of nucleic acid in a lipid nanoparticle formulation may also depend on the size, composition, desired target, and other characteristics of lipid nanoparticle formulation. The relative amounts of payload, e.g., mRNA or other macromolecule and other elements (e.g., lipids) may also vary. In some embodiments, the wt/wt ratio of the lipid component to a nucleic acid, such as an mRNA, in a nanoparticle composition may be from about 5:1 to about 50:1, such as 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, and 50:1. For example, the wt/wt ratio of the lipid component to a nucleic acid, such as an mRNA may be from about 10:1 to about 40:1. The amount of nucleic acid in a nanoparticle composition

may, for example, be measured using absorption spectroscopy (*e.g.*, ultraviolet-visible (UV-vis) spectroscopy).

[0157] The lipid nanoparticle formulations can comprise a nucleic acid in a concentration from approximately 0.1 mg/ml to 2 mg/ml such as, but not limited to, 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml, 1.3 mg/ml, 1.4 mg/ml, 1.5 mg/ml, 1.6 mg/ml, 1.7 mg/ml, 1.8 mg/ml, 1.9 mg/ml, 2.0 mg/ml or greater than 2.0 mg/ml.

[0158] Preferably, the one or more nucleic acids (*e.g.* mRNAs), lipids, and amounts thereof may be selected to provide a specific N:P ratio. The N:P ratio of the composition refers to the molar ratio of nitrogen atoms in one or more lipids to the number of phosphate groups in a nucleic acid (e.g., an mRNA). In general, a lower N:P ratio is preferred. The one or more mRNA, lipids, and amounts thereof may be selected to provide an N:P ratio from about 2:1 to about 8:1, such as 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, and 8:1. In certain embodiments, the N:P ratio may be from about 2:1 to about 5:1. In preferred embodiments, the N:P ratio may be about 4:1. In other embodiments, the N:P ratio is from about 5:1 to about 8:1. For example, the N:P ratio may be about 5.0:1, about 5.5:1, about 5.67:1, about 6.0:1, about 6.5:1, or about 7.0:1. See, for example, US Patent No. 11,318,213 and U.S, Published Application Nos. 2020/0163878 each of which is incorporated by reference.

[0159] In some embodiments of the nucleic acid-containing lipid composition, nucleic acid is complexed to the exterior of the lipid complex (e.g., liposomes, lipid nanoparticles). In some embodiments, the compositions have from about 20% to about 50% of the nucleic acid complexed to the exterior of the lipid complex. In other embodiments, the compositions have about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, or about 80% of the nucleic acid complexed to the exterior of the lipid complex. Exterior complexation of a nucleic acid can be measured by methods know in the art, such as in Blakney et al. (2019) Gene Therapy 26:363-372.

[0160] In some embodiments of the nucleic acid-containing lipid composition, nucleic acid is complexed on the interior of the lipid complex (e.g., liposomes, lipid nanoparticles). In some embodiments, the compositions have an encapsulation efficiency from about 75% to about 95%,

or from about 85% to about 90%. In some examples, the encapsulation efficiency is from about 75% to about 100%. In some examples, the encapsulation efficiency is from about 75% to about 95%. In some examples, the encapsulation efficiency is from about 75% to about 90%. In some examples, the encapsulation efficiency is from about 75% to about 85%. In some examples, the encapsulation efficiency is from about 75% to about 80%. In some examples, the encapsulation efficiency is from about 80% to about 95%. In some examples, the encapsulation efficiency is from about 80% to about 90%. In some examples, the encapsulation efficiency is from about 80% to about 85%.

Exosomes, exosome lipids

[0161] The lipid compositions provided herein can also be combined with one or more exosomes, or biological materials (*e.g.*, lipids, proteins, nucleic acids, or the like) derived or purified from exosomes.

[0162] The term "exosome" refers to the small membrane vesicles secreted by most cells that contain cell specific payloads of proteins, lipids and, genetic material and other biomolecules that are transported to other cells in different location of the tissue. Exosomes can be considered liposomal particles. Exosomes or lipid mixtures obtained therefrom, can be used in combination with other transfection agents or helper lipid mixtures. Exosomes are also referred to as microvesicles, epididimosomes, argosomes, exosome-like vesicles, microparticles, promininosomes, prostasomes, dexosomes, texosomes, archeosomes and oncosomes.

[0163] Examples of lipid constituents isolated from exosomes useful in the compositions provided herein include, but are not limited to, Lyso-PC (non-limiting examples of which C-18, C-16, C-14 and mixture), Lyso-bisphospahtidic acid (non-limiting example of which is C-18, C-16 and C-14), sphingomyelin, ceramides (non-limiting examples C-8- C-24), disaturated PC (non-limiting examples DSPC, DPPC, DMPC and others where Cn (n= 8 – 25)), diunsaturated PC-MIX (non-limiting examples of which are DOPC, DP(db)PC) phosphatidyl serine (PS), phosphatidyl inositol (PI)), disaturated PE non-limiting example, DSPE, DPPE, DMPE), di-unsaturated PE-MIX (non-limiting example DOPE DP(db)PE), phosphatidyl glycerol (PG) (non-limiting examples of which are C-18 – C-22), cholesterol, and diglycerides, such as cardiolipin.

[0164] Exemplary compositions can include, for example, a lipid of Formula (I) and one or more exosomes (and optionally a payload); a lipid of Formula (I), and one or more exosomes, and one or more neutral lipids (and optionally a payload); a lipid of Formula (I), and one or more exosomes, one or more neutral lipids, and one or more stabilizing agents (and optionally a payload); a lipid of Formula (I), and one or more exosomes, and one or more neutral lipids, optionally one or more stabilizing agents, and optionally one or more cell penetrating peptides (and optionally a payload).

[0165] Other exemplary compositions include, for example; a lipid of Formula (I), and one or more biological materials derived or purified from exosomes (and optionally a payload); a lipid of Formula (I), and one or more biological materials derived or purified from exosomes, and one or more neutral lipids (and optionally a payload); a lipid of Formula (I), and one or more biological materials derived or purified from exosomes, one or more neutral lipids, and one or more stabilizing agents (and optionally a payload); a lipid of Formula (I), and one or more biological materials derived or purified from exosomes, and one or more neutral lipids, optionally one or more stabilizing agents, and optionally one or more cell penetrating peptides (and optionally a payload).

[0166] Other exemplary compositions may include a lipid of Formula (I), and one or more biological materials derived or purified from viruses, such a viral lipids, viral proteins or a viral nucleic acid fragment, optionally with one or more stabilizing agents.

[0167] Also contemplated are any mixtures of combination of the above listed ionizable lipids, neutral lipids, exosomes, and lipid mixtures isolated from exosomes.

Ionizable lipids

[0168] The lipid compositions provided herein can include one or more cationinc/ionizable lipids, in addition to the lipid of Formula (I). For example, some lipid compositions include a cationic/ionizable lipid selected from the group consisting of DOTMA, DOTAP, DMRIE, DC-Chol, DDAB, DOSPA, DOSPER, DOGS, TMTPS, TMTOS, TMTLS, TMTMS, DHDMS, HDMS TMDOS,, N-1-dimethyl-N-1-(2,3-diaoleoyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-

diaoleoyloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, L-spermine-5-carboxyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine, 3,5-(N,N-di-lysyl)-diaminobenzoyl-glycyl-3-(DL-1,2-dipalmitoyl-dimethylami nopropyl-β-hydroxyethylamine), L-Lysine-bis(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, L-Lysine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-alkylamino)-2-hydroxypropyl)piperazine, L-Lysine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxypropyl]piperazine, L-Ornithine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4,-bis[(3-amino-2-hydroxypropyl)-oleylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxypropyl)-palmitylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxypropyl)-myristylamino]-butane-2,3-diol, 1,4-bis[(3-oleylamino)propyl]piperazine, L-Arginine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, bis[(3-(3-aminopropyl)-myristylamino)2-hydroxypropyl]piperazine, L-Arginine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Serine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]piperazine, Glycine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, Sarcosine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Histidine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, cholesteryl-3β-carboxyl-amidoethylenetrimethylammonium iodide, 1,4-bis[(3-myristylamino)propyl]piperazine, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl carboxylate iodide, cholesteryl-3β-carboxyamidoethyleneamine, cholesteryl-3β-oxysuccinamidoethylenetrimethylammonium iodide, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl-3β-oxysuccinate iodide, 2-[(2-trimethylammonio)-ethylmethylamino] ethyl-cholesteryl-3β-oxysuccinate iodide, 3β[N-(N', N'-dimethylaminoethane)carbamoyl]cholesterol, and 3β-[N-(polyethyleneimine)-carbamoyl] cholesterol,1,4-bis[(3-palmitylamino)propyl]piperazine, L-Ornithylglycyl-N-(1-heptadecyloctadecyl)glycinamide, N^2,N^5 -Bis(3-aminopropyl)-L-ornithylglycyl-N- (1-heptadecyloctadecyl)glycinamide, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-alkylamino)-2-hydroxypropyl]piperazine, N^2-[N^2,N^5 -Bis(3-aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L-

glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dioctadecyl-L-α-glutamine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleylamino)2-hydroxypropyl]piperazine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dioctadecyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]- N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioctadecyl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5 -Bis(3-aminopropyl)-L-ornithyl]-N,N-diolyl-L-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-glutamine,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)-2-hydroxypropyl]piperazine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]- N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioleyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-oleylamino)propyl]piperazine, N^2-[N^2,N^5 -Bis(3-aminopropyl)-L-ornithyl]-N,N-dipalmityl-L-glutamine,N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dipalmityl-L-α-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dipalmityl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]- N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dipalmityl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5 -Bis(3-aminopropyl)-L-ornithyl]-N,N-dimyristyl-L-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-asparagine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmitylamino)-2-hydroxypropyl]piperazine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dimyristyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-myristylamino)propyl]piperazine, N^2-[N^2,N^5 -Bis(3-aminopropyl)-L-ornithyl]-N,N-dilaureyl-L-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dilaureyl-L-α-glutamine, N^2-[N^2,N^5 -Bis(aminopropyl)-L-ornithyl]-N-N-dilaureyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]- N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dilaureyl-L-glutaminyl]-L-glutamic acid, 3-[N',N''-bis(2-tertbutyloxycarbonylaminoethyl)guanidino]-N,N-dioctadec-9-enylpropionamide, 3-[N',N''-bis(2-tertbutyloxycarbonylaminoethyl)guanidino]-N,N-dipalmitylpropionamide, 3-[N',N''-bis(2-tertbutyloxycarbonylaminoethyl)guanidino]-N,N-dimyristylpropionamide, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)propyl]piperazine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleylamino)propyl]piperazine, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-diolylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-

dipalmitylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-dimyristylaminopropane, 1,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)propyl]piperazine, [(3-aminopropyl)-bis-(2-tetradecyloxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-oleyloxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-palmityloxyethyl)]methyl ammonium bromide, Oleoyl-2-hydroxy-3-N,N-dimethyamino propane, 2-didecanoyl-1-N,N-dimethylaminopropane, palmitoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dipalmitoyl-1-N,N-dimethylaminopropane, myristoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dimyristoyl-1-N,N-dimethylaminopropane, (3-Amino-propyl)-›4-(3-amino-propylamino)-4-tetradecylcarbamoyl-butylcarbamic acid cholesteryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino-4-carbamoylbutylcarbamic acid cholesteryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino)-4-(2-dimethylamino-ethylcarbamoy l)-butylcarbamic acid cholesteryl ester, Spermine-5-carboxyglycine (N'-stearyl-N'-oleyl) amide tetratrifluoroacetic acid salt, Spermine-5-carboxyglycine (N'-stearyl-N'-elaidyl) amide tetratrifluoroacetic acid salt, Agmatinyl carboxycholesterol acetic acid salt, Spermine-5-carboxy-β-alanine cholesteryl ester tetratrifluoroacetic acid salt, 2,6-Diaminohexanoeyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, 2,4-Diaminobutyroyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, N,N-Bis (3-aminopropyl)-3-aminopropionyl β-alanine cholesteryl ester tristrifluoroacetic acid salt., [N,N-Bis(2-hydroxyethyl)-2-aminoethyl]aminocarboxy cholesteryl ester, Stearyl carnitine ester, Palmityl carnitine ester, Myristyl carnitine ester, Stearyl stearoyl carnitine ester chloride salt, L-Stearyl Stearoyl Carnitine Ester, Stearyl oleoyl carnitine ester chloride, Palmityl palmitoyl carnitine ester chloride, Myristyl myristoyl carnitine ester chloride, L-Myristyl myristoyl carnitine ester chloride, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmitylamino)propyl]piperazine, N-(3-aminopropyl)-N,N'-bis-(dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(myristyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-myristyloxyethyl)-piperazinium bromide, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxy-propyl]piperazine, and

1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxy-propyl]piperazine, KL22, KL25, 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA or MC3), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)- -octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z- ,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-- octadeca-9,12-dien-1-yloxy]prop an-1-amine (Octyl-CLinDMA (2S)).

[0169] In some embodiments, the ionizable lipid includes one or more cationic lipid selected from GeneIn™, LipofectAmine™ 2000, LipofectAmine™, Lipofectin®, DMRIE-C, CellFectin® (Invitrogen), Oligofectamine® (Invitrogen), LipofectAce® (Invitrogen), Fugene® (Roche, Basel, Switzerland), Fugene® HD (Roche), Transfectam® (Tranfectam, Promega, Madison, WI), Tfx-10® (Promega), Tfx-20® (Promega), Tfx-50® (Promega), Transfectin™ (BioRad, Hercules, CA), SilentFect™ (Bio-Rad), Effectene® (Qiagen, Valencia, CA), DC-chol (Avanti Polar Lipids), GenePorter® (Gene Therapy Systems, San Diego, CA), DharmaFect 1® (Dharmacon, Lafayette, CO), DharmaFect 2® (Dharmacon), DharmaFect 3® (Dharmacon), DharmaFect 4® (Dharmacon), Escort™ III (Sigma, St. Louis, MO), Escort™ IV (Sigma), DOTMA, DOTAP, DMRIE, DC-Chol, DDAB, DOSPA, DOSPER, DOGS, TMTPS, TMTOS, TMTLS, TMTMS, TMDOS, N-1-dimethyl-N-1-(2,3-diaoleoyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxy-propyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diaoleoyl-oxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxy-propyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalm-ityloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, L-spermine-5-carboxyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine, 3,5-(N,N-di-lysyl)-diamino-benzoyl-glycyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine), L-Lysine-bis(O,O'-oleoyl-β-hydroxy-ethyl)amidedihydrochloride, L-Lysine-bis-(O,O'-palmitoyl-β-

hydroxyethyl)amidedihydrochloride, 1,4-bis[(3-(3-aminopropyl)-alkylamino)-2-hydroxypropyl)-piperazine, L-Lysine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, L-Ornithine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4,-bis[(3-amino-2-hydroxypropyl)-oleylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxy-propyl)-palmitylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxypropyl)-myristylamino]-butane-2,3-diol, 1,4-bis[(3-oleylamino)propyl]-piperazine, L-Arginine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, bis[(3-(3-aminopropyl)-myristylamino)2-hydroxy-propyl]piperazine, L-Arginine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Serine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]piperazine, Glycine-bis-(O,O'-palmitoyl-β-hydroxy-ethyl)amide dihydrochloride, Sarcosine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Histidine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, cholesteryl-3β-carboxyl-amidoethylenetrimethylammonium iodide, 1,4-bis[(3-myristyl-amino)-propyl]-piperazine, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl carboxylate iodide, cholesteryl-3β-carboxyamidoethyleneamine, cholesteryl-3β-oxysuccinamido-ethylene-trimethylammonium iodide, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl-3β-oxysuccinate iodide, 2-[(2-trimethylammonio)-ethylmethyl-amino]ethyl-cholesteryl-3β-oxysuccinate iodide, 3β[N-(N', N'-dimethylamino-ethane)-carbamoyl]cholesterol, and 3β-[N-(polyethyleneimine)-carbamoyl] cholesterol,1,4-bis[(3-palmitylamino)propyl]piperazine, L-Ornithylglycyl-N-(1-heptadecyloctadecyl)-glycinamide, N^2,N^5-Bis(3-aminopropyl)-L-ornithyl-glycyl-N-(1-heptadecyloctadecyl)-glycinamide, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-alkyl-amino)-2-hydroxypropyl]-piperazine, N2-[N2,N5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L-glutamine,N2-[N2,N5-Bis(aminopropyl)-L-ornithyl]-N-N-dioctadecyl-L-α-glutamine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleylamino)2-hydroxypropyl]piperazine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dioctadecyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethyl-ethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioctadecyl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-diolyl-L-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-glutamine,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)-2-hydroxy-propyl]piperazine, N^2-

[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioleyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-oleyl-amino)-propyl]piperazine, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dipalmityl-L-glutamine,N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dipalmityl-L-α-glutamine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dipalmityl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)-carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dipalmityl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dimyristyl-L-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-asparagine, 1,4-bis[(3-(3-amino-2-hydroxy-propyl)-palmitylamino)-2-hydroxypropyl]-piperazine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethyl-ethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dimyristyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-myristyl-amino)-propyl]piperazine, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dilaureyl-L-glutamine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dilaureyl-L-α-glutamine, N^2-[N^2,N^5–Bis-(amino-propyl)-L-ornithyl]-N-N-dilaureyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)-carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dilaureyl-L-glutaminyl]-L-glutamic acid, 3-[N',N"-bis(2-tertbutyloxycarbonyl-amino-ethyl)guanidino]-N,N-dioctadec-9-enylpropionamide, 3-[N',N"-bis(2-tertbutyloxy-carbonylamino-ethyl)guanidino]-N,N-dipalmitylpropionamide, 3-[N',N"-bis(2-tertbutyl-oxycarbonyl-aminoethyl)guanidino]-N,N-dimyristylpropionamide, 1,4-bis[(3-(3-amino-propyl)-palmityl-amino)propyl]piperazine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleyl-amino)propyl]piperazine, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-diolylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-dipalmitylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-dimyristylaminopropane, 1,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)-propyl]piperazine, [(3-aminopropyl)-bis-(2-tetradecyl-oxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-oleyloxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-palmityloxyethyl)]methyl ammonium bromide, oleoyl-2-hydroxy-3-N,N-dimethyamino propane, 2-didecanoyl-1-N,N-dimethylaminopropane, palmitoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dipalmitoyl-1-N,N-dimethylamino-propane, myristoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dimyristoyl-1-N,N-

dimethylaminopropane, (3-amino-propyl)-›4-(3-amino-propylamino)-4-tetradecyl-carbamoyl-butylcarbamic acid cholestryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino-4-carbamoylbutylcarbamic acid cholestryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino)-4-(2-dimethylamino-ethylcarbamoy-l)-butylcarbamic acid cholesteryl ester, Spermine-5-carboxyglycine (N'-stearyl-N'-oleyl) amide tetratrifluoro-acetic acid salt, Spermine-5-carboxyglycine (N'-stearyl-N'-elaidyl) amide tetratri-fluoroacetic acid salt, Agmatinyl carboxycholesterol acetic acid salt, Spermine-5-carboxy-β-alanine cholesteryl ester tetratrifluoroacetic acid salt, 2,6-Diaminohexanoeyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, 2,4-Diaminobutyroyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, N,N-Bis (3-aminopropyl)-3-aminopropionyl β-alanine cholesteryl ester tristrifluoroacetic acid salt, [N,N-Bis(2-hydroxyethyl)-2-aminoethyl]aminocarboxy cholesteryl ester, Stearyl carnitine ester, Palmityl carnitine ester, Myristyl carnitine ester, Stearyl stearoyl carnitine ester chloride salt, L-Stearyl Stearoyl Carnitine Ester, Stearyl oleoyl carnitine ester chloride, Palmityl palmitoyl carnitine ester chloride, Myristyl myristoyl carnitine ester chloride, L-Myristyl myristoyl carnitine ester chloride, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmityl-amino)propyl]-piperazine, N-(3-aminopropyl)-N,N'-bis-(dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(myristyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-myristyloxyethyl)-piperazinium bromide, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxy-propyl]piperazine, or 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]-piperazine, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-aminopropyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-amino-propyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-aminopropyl)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diamino-butane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(5-carboxamido-spermine)-diaminobutane, 2,3-dipalm-

itoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(5-carboxamidospermine)-diaminobutane, 2,3-dimyrist-oleoyloxy-1,4-N,N'-dimethyl-N,N'-di(5-caqrboxamidospermine)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diamino-butane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dipalmitoleoyl-oxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dioleyloxy-N,N'-dimethyl-1,4 -diaminobutane, 2,3-dipalmitoleoyloxy-N,N'-dimethyl-1,4-diaminobutane, 2,3-dimyrist-oleoyloxy-N,N'-dimethyl-1,4-diaminobutane; PAMAM dendrimers, NH3 core dendrimers, ethylenediamine core dendrimers, polyethylenimine, and polyethylenimine conjugates.

[0170] Preferably, the lipid of Formula (I), or the combination of the lipid of Formula (I) with one or more cationic/ionizable lipids, is present at about 5-80 mol% of the lipid composition. For example, some lipid compositions include less than 50 mol% Formula (I), or combination of Formula (I) and one or more additional cationic/ionizable lipids. Other lipid compositions include more than 50 mol% Formula (I), or combination of Formula (I) and one or more additional cationic/ionizable lipids.

[0171] Accordingly, some lipid compositions include a lipid of Formula (I), or a combination of Formula (I) and one or more cationic/ionizable lipids at 15-80 mol%, a sterol at 20-60 mol%, a stabilizing agent at 0.5-5 mol%, and a phospholipid at 1-40 mol% of the lipid composition. An exemplary lipid composition can include about 20-60 mol % Formula (I) lipid or combination of Formula (I) lipid and one or more additional cationic/ionizable lipids, about 5-25 mol % phospholipid, about 25-55 mol% sterol or sterol derivative; and about 0.5-15 mol% stabilizing agent. Another exemplary lipid composition includes a about 50 mol % lipid of Formula (I) or combination of lipid of Formula (I) and one or more cationic/ionizable lipids, about 1.5 mol% stabilizing agent, about 38.5 mol% sterol or sterol derivative, and about 10 mol% phospholipid. Another exemplary lipid composition comprises about 55% lipid of Formula (I) or combination of lipid of Formula (I) and one or more cationic/ionizable lipids, about 2.5 mol % stabilizing agent, about 32.5 mol % sterol or sterol derivative, and about 10 mol % phospholipid.

Neutral lipids

[0172] Advantageously, in addition to the cationic/ionizable lipids, the lipid compositions include one or more neutral co-lipids, although the skilled artisan will recognize that other co-lipids may be used. The neutral lipid may be, for example, selected from the group consisting of a sterol or sterol derivative, a phospholipid, or a combination thereof.

[0173] The neutral lipid can be present at about 5-60 mol% of the overall lipid formulation. In some embodiments, neutral lipid(s) are present from about 15-50 mol%, e.g., 25-40 mol %. In certain embodiments, the amount of the neutral lipid in the lipid composition disclosed herein is at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 mol % of the overall lipid formulation.

[0174] In some embodiments, the lipid composition includes a neutral lipid, and the neutral lipid includes cholesterol. In some embodiments, the neutral lipid includes sterols, or lipids containing sterol moieties ("sterol derivatives"). As defined herein, "sterols" are a subgroup of steroids consisting of steroid alcohols. Exemplary sterols and lipids containing sterol moieties useful in the lipid composition formulations provided herein include, but are not limited to cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, hopanoids, phytosterols, steroids, and mixtures thereof. In some embodiments, the structural lipid is a sterol. Some lipid composition formulations provided herein include a sterol or sterol derivative. The sterols or sterol derivatives can be present at about 5-60 mol% of the overall lipid composition formulation. Advantageously, the sterol or sterol derivatives are present from about 15-50 mol%, e.g., 25-40 mol %. Preferably, the amount of the sterol (such as cholesterol) or sterol derivative in the lipid composition disclosed herein is at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 mol % of the overall lipid formulation. Some lipid composition formulations provided herein do not include a sterol or sterol derivative.

[0175] In some embodiments, the compositions include a structural lipid in a concentration of 14-50 mol% of the composition, excluding any payload, if present. In some embodiments, the structural lipid is selected from the group consisting of: cholesterol, fecosterol, sitosterol,

ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, hopanoids, phytosterols, steroids, and any combination thereof.

[0176] In some embodiments, the neutral lipid includes a phospholipid. For example, in some embodiments, the neutral lipid is a phospholipid such as dioleoylphosphatidylethanolamine (DOPE). In some embodiments, the neutral lipid includes N-Palmitoyl phosphatidylethanolamine. In some embodiments, the neutral lipid includes N-Oleoyl-DPPE. In some embodiments, the neutral lipid includes diphytanoylphosphatidylethanolamine (DPhPE). In some embodiments, the neutral lipid includes Lyso-PE (1-acyl-2-hydroxy-sn-glycero-3-phosphoethanolamine). In some embodiments, the neutral lipid includes Lyso-PC (1-acyl-3-hydroxy-sn-glycero-3-phosphocholine). In some embodiments, the neutral lipid includes distearoylphosphatidylcholine (DSPC). In some embodiments, the neutral lipid includes dioleoylphosphatidylcholine (DOPC). In some embodiments, the neutral lipid includes dipalmitoylphosphatidylcholine (DPPC). In some embodiments, the neutral lipid includes palmitoyloleoylphosphatidylcholine (POPC). In some embodiments, the neutral lipid includes palmitoyloleoyl-phosphatidylethanolamine (POPE) and dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal). In some embodiments, the neutral lipid includes dipalmitoyl phosphatidyl ethanolamine (DPPE). In some embodiments, the neutral lipid includes dimyristoylphosphoethanolamine (DMPE). In some embodiments, the neutral lipid includes distearoyl-phosphatidylethanolamine (DSPE). In some embodiments, the neutral lipid includes 16-O-monomethyl PE. In some embodiments, the neutral lipid includes 16-O-dimethyl PE. In some embodiments, the neutral lipid includes 18-1-trans PE. In some embodiments, the neutral lipid includes 1-stearoyl-2-oleoyl-phosphatidyethanol amine (SOPE). In some embodiments, the neutral lipid includes 1,2-dioleoyl-sn-glycero-3-phophoethanolamine (trans DOPE). In some embodiments, the neutral lipid includes any combinations thereof.

[0177] Exemplary phospholipids useful in the compositions disclosed herein include, but are not limited to, dioleoylphosphoethanolamine (DOPE), diphytanolphosphatidylethanolamine (DPhPE), Lyso-PE (1-acyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine), Lyso-PC (1-acyl-3-hydroxy-sn-glycero-3-phosphocholine), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine

(POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), 1,2-dioleoyl-sn-glycero-3-phophoethanolamine (trans DOPE), 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC), dilinoleoylphosphocholine (DLPC), dimyristoylphosphocholine (DMPC), diundecanoylphosphocholine (DUPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, and lysophosphatidylethanolamine (LPE), or any combination thereof.

[0178] When present, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 0.5 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 1 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 2 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 3 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 4 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 5 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 10 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 12 mol %.

In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 15 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 16 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 17 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 18 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 19 mol %. In some embodiments, the amount of the phospholipid in the lipid composition formulations disclosed herein is at least about 20 mol %.

PEG-modified lipids

[0179] The lipid compositions provided herein can also include a stabilizing agent, such as a stabilizing lipid. Stabilizing lipids can be neutral lipids, or they can be charged. Stabilizing lipids that can advantageously be used in the formulations provided herein include, but are not limited to, polyethylene glycol (PEG)-modified lipids. Non-limiting examples of PEG-lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids. For example, a PEG lipid can be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid. Other exemplarily PEGylated lipids include DSPE-PEG_{2000} (distearoyl-poly(ethylene glycol)), PEG_{2000}-DSPE-PEG_{500}, 1,2-distearyloxypropyl-3-amine-PEG_{2000}, hexadecylcarbamoylmethyl hexadecanoate-PEG_{2000}, cholesteryl hemisuccinate-PEG_{2000}, photocleavable cholesteryl-PEG_{2000}, cholesterol-hyperbranched polyglycerol, poly(2-methyl-2-oxazoline) (PMOZ)- or poly(2-ethyl-2-oxazoline) (PEOZ)-DSPE, poly(hydroxyethyl-l-asparagine)-succinyldioctadecylamine, DSPE-poly(2-tert-butoxy-N-(2-(methacryloyloxy)ethyl)-N,N-dimethyl-2-oxoethanamonium). In some embodiments, the lipid compositions do not include a PEGylated lipid.

[0180] Other stabilizing agents useful in the compositions disclosed herein include, e.g., polyglycol lipids, oxyethylene alkyl ethers, diblock polyoxyethylene ether co-polymers, triblock polyoxyethylene alkyl ethers co-polymers, and amphiphilic branched polymers. In embodiments, stabilizing agent can be polyoxyethylene (20) oleyl ether, polyoxyethylene (23) lauryl ether,

polyoxyethylene (40) stearate ("Myrj52"), poly(propylene glycol)11-block-poly(ethylene glycol)16-block-poly(propylene glycol)11, poly(propylene glycol)12-block-poly(ethylene glycol)28-block-poly(propylene glycol)12, polysorbate 80 (also known as Tween 80, IUPAC name 2-[2-[3,4-bis(2-hydroxyethoxy)oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl octadec-9-enoate), Myrj52 (Polyoxyethylene (40) stearate), Brij™ S10 (Polyoxyethylene (10) stearyl ether), BRIJ™ L4 = Polyoxyethylene (4) lauryl ether; BRIJ™ S20= Polyoxyethylene (20) stearyl ether; BRIJ™ S35= Polyoxyethylene (23) lauryl ether; TPGS 1000 =D-α-Tocopherol polyethylene glycol 1000 succinate; Tween 20/Polysorbate 80/ Tridecyl-D-maltoside in equal ratios, and combinations thereof.

[0181] In certain compositions, the stabilizing agent (e.g., PEGylated lipid or other stabilizing agent), is present at about 0.1 - 5 mol% of the lipid composition. For example, in some compositions, the stabilizing agent is present at about 0.5 mol%, 1 mol%, 1.5 mol%, 2 mol%, 2.5 mol %, 3 mol%, 3.5 mol %, 4 mol %, 4.5 mol%, 5 mol%, or any value in between, of the lipid composition. In other examples, the stabilizing agent is present at about 0.5 mol% to about 5 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 0.5 mol% to about 4 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 0.5 mol% to about 3 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 0.5 mol% to about 2 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 0.5 mol% to about 1 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 1 mol% to about 5 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 1 mol% to about 4 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 1 mol% to about 3 mol% of the lipid composition. In other examples, the stabilizing agent is present at about 1 mol% to about 2 mol% of the lipid composition.

[0182] In some embodiments, the compositions include a stabilizing agent in a concentration of 0.1-10 mol% of the composition, excluding any payload, if present.

[0183] In some embodiments, stabilizing agent is selected from the group consisting of: a surfactant, a neutral lipid, a polymer-conjugated lipid, polyethylene glycol, a phospholipid, and any combination thereof. In some embodiments, the stabilizing agent is a PEG-modified lipid. Exemplary PEG-modified lipids include, but are not limited to, a PEG-modified

phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-ceramide conjugate, a PEG-modified dialkylamine, a PEG-modified 1,2-diacyloxypropan-3-amine, and any combination thereof. In some embodiments, the PEG-modified lipid is selected from PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE, and any combination thereof.

[0184] In some embodiments, the stabilizing agent comprises one or more phospholipids selected from the group consisting of: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin.

[0185] In some embodiments, the formulations may also include one or more lipids derived from viral capsids, e.g., from enveloped viruses.

Polyamine Components / Polymers

[0186] Other formulations may also include one or more polyamine transfection agent or polymer, such as dense star dendrimers, PAMAM dendrimers, NH_3 core dendrimers, ethylenediamine core dendrimers, dendrimers of generation 5 or higher, dendrimers with substituted groups, dendrimers comprising one or more amino acids, grafted dendrimers, activated dendrimers, polyethylenimine, polyethylenimine conjugates, polylysine, polyarginine, polyornithine, histone, and any combination thereof. In some embodiments, the polymer is a linear or branched PEI.

Transfection Enhancing Agents

[0187] Still other formulations may include transfection enhancing agents such as a fusion agent (such as an endosomal release agent), a cell surface ligand and/or a nuclear localization agent such as a nuclear receptor ligand peptide, Examples of transfection enhancing agents include, but are not limited to, reovirus-related fusogenic peptides (see, e.g., WO07/130073, which is hereby incorporated by reference in its entirety), enveloped and nonenveloped virus particles or inactivated virus, particles or proteins form these viruses, insulin, a transferrin, epidermal growth factor, fibroblast growth factor, a cell targeting antibody, a lactoferrin, a fibronectin, an adenovirus penton base, Knob, a hexon protein, a vesicular stomatitis virus glycoprotein, a Semliki Forest Virus core protein, a influenza hemagglutinin, a hepatitis B core protein, an HIV Tat protein, a herpes simplex virus VP22 protein, a histone protein, a arginine rich cell permeability protein, a high mobility group protein, and invasin protein, and internalin protein, an endotoxin, a diphtheria toxin, a shigella toxin, a melittin, a magainin, a gramicidin, a cecrophin, a defensin, a protegrin, a tachyplesin, a thionin, a indolicidin, a bactenecin, a drosomycin, an apidaecin, a cathelicidin, a bactericidal-permeability-increasing protein, a nisin, a buforin, and fragments thereof.

[0188] The compositions described herein may include one or more transfection enhancing agents (e.g., a polycationic nucleic acid binding moiety). In some embodiments, the transfection enhancing agent selected from an endosomal release agent, a cell surface ligand, a nuclear localization agent, a cell-penetrating peptide, a fusogenic peptide, and any combination thereof. In some embodiments, the one or more transfection enhancing agents includes an amphipathic peptide.

[0189] The lipid nanoparticle compositions provided herein can also be combined with one or more exosomes, or biological materials (*e.g.*, lipids, proteins, nucleic acids, or the like) derived or purified from exosomes.

[0190] Other cell penetrating peptides useful in the compositions provided herein include those provided in Table II (*See* WO 2023/018990, which is hereby incorporated by reference in its entirety), below:

TABLE II

SEQ ID No.	Sequence
1	GYSTPPKKKRKVEDP
2	GYSTPPKTRRRP
3	GYSTPGRKKR
4	GYSTPRRNRRRRW
5	PDEVKRKKKPPTSYG
6	PRRRTKPPTSYG
7	RKKRGPTSYG
8	WRRRRNRRPTSYG
9	GYGPPKKKRKVEAPYKA
10	PAAKRVKLD
11	RQRRNELKRSP
12	KRPAATKKAGQAKKKK
13	VRKKRKTEEESPLKDKDAKKSKQE
14	RLRRDAGGRGGVYEHLGGAPRRRK
15	KRKGDEVDGVDECAKKSKK
16	NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY
17	GGKRTADGSEFESPKKARKVEAYPKAW
18	GGKRTADGSEFESPKKKRAVEAYPKAW
19	GGKRTADGSEFESPKKKAKVEAYPKAW
20	GGKRTADGSEFESPKKKRKVEAPYKAWK
21	GGKRTADGSEFESPKKKRKVEYKAWK
22	GYGPAAKRVKLDEAYPKAWK
23	GGKRTADGSEFEPAAKRVKLDEAYPKAWK
24	GTGPKKKRKVGGGGYGPKKKRLVG
25	KRPAATKKAGQAKKKKLEAYPKAWK
26	ATKGTKRSYEQMETGE
27	GKWERKPIRCAS
28	GYGKRTADSQHSTPPKKKRKVEAPYKAWK
29	KRTADSQHSTPPKKKRKVEAPYKAWK
30	GYGPPKKKRKVEAPYKAWKWAKYPAMRRAHHRRRRASHRRRTTTGT
31	GYGPPKKKRKVEAPYKAWKRGARRYSKMKRRRRRVARRHRRRP
32	FWGYGYGPPKKKRKVEAPYKAWK

SEQ ID No.	Sequence
33	GKPSSDDEATADSQHSTPPKKKERKVED
34	GKPTADDQHSTPPKKKRKVED
35	GGKRTADGSEFESPKKARKVEAYPKAK
36	EKIRLRPGRKKRYRLKHL
37	PEGTRQARRNRRRRWRKR
38	PEGTRQPRRNRRRRWRKR
39	GVKRSYGAARGDDRRRPNVVAPYKAW
40	KSVPNRTRTYIKLKRLRFKGAPYKAW
41	EMRRRREEEGLQLRKQKREEQLFKRRN
42	FEAALAEALAEALA
43	Ac-LARLLPRLLARL-$NHCH_3$
44	GLLEELLELLEELWEELLEG
45	GWEGLIEGIEGGWEGLIEG
46	GLFEALAEFIEGGWEGLIEG
47	GLFEALLELLESLWELLLEA
48	GGYCLEKWMIVASELKCFGNTA
49	GGYCLTRWMLIEAELKCFGNTAV
50	WEAALAEALAEALAEHLAEALAEALEALAA
51	GLFGAIAGFIENGWEGMIDGWYG
52	GIGAVLKVLTTGLPALISWIKRKRQQ
53	GRKKRRQRRRPPQ
54	RQIKIWFQNRRMKWKK
55	GWTLNSAGYLLGKINLKALAALAKKIL
56	WEAKLAKALAKALAKHLAKALAKALKACEA
57	GLFKALLKLLKSLWKLLLKA
58	GLFRALLRLLRSLWRLLLRA
59	GLFEALLELLESLYELLLEA
60	GLFEALEELWEA
61	GLFLLEEWLE
62	GLFLLEEWLEK
63	GLFEALLELLESLWELLLEAK
64	Suc-GLFKLLEEWLE
65	Suc-GLFKLLEEWLEK
66	GLFEAIAEFIEGGWEGLIEG

SEQ ID No.	Sequence
67	GLFKAIAKFIKGGWKGLIKG
68	IRFKKTKLIASIAMALC
69	ALAGTIIAGASLTFQVLDKVIEELGKVSRK
70	GLFEAIEGFIENGWEGMIDGWYG
71	GYICRRARGDNPDDRCT
72	GLFEAIAEFIEGGWEGLIEGCA
73	GLFHAIAHFIHGGWHGLIHGWWYG
74	RRRQRRKKRGGDIMGEWGNEIFGAIAGFLG
75	GLFEAIADFIENGWEGMIDGGG
76	ALAGTIIAGASLTFQVLDKVIEELGKVSRKK
77	IRFKKTKLIASIAMA
78	GLWHLLLHLWRRLLRLLR
79	KKIMLLLMTLLLVSLPLAQEQ
80	GLFEALLELLESLWELLLEAWYG
81	RLLRLLLRLWRRLLRLLR
82	LLELELLELELLLELELLELELLLEL
83	GLFEALLELLESLWELLLEARRRRRRRR
84	GLFEALLELLESLWELLLEARRRRRR
85	GLFEALLELLESLWELLLEAKKKKKKKK
86	GLFEALLELLESLWELLLEAKKKKKK
87	GLFEALLELLESLWELLLEAKK
88	GLFEALLELLESLWELLLEAKKKK
89	GLFEALLELLESLWELLLEAEE
90	GLFEALLELLESLWELLLEAEEEE
91	GLFEALLELLESLWELLLEAEEEEEE
92	GLFEALLELLESLWELLL
93	PLSSIFSRIGDPRGARRYAKMKRRRRRVARRHRRRP
94	GPFHYFQFLFPPV
95	GSSSWWQRWWPPW
96	RRRQRRKKR
97	KKKK
98	KKKKKK
99	KKKKKKKK
100	KKKKKKKKKK
101	KKKKKKKKKKKK

SEQ ID No.	Sequence
102	KKKKKKKKKKKKKKK
103	KKKKKKKKKKKKKKKKKKK
104	KKKKKKKKKKKKKKKKKKKKKKK
105	RRRR
106	RRRRRR
107	RRRRRRRR
108	RRRRRRRRRR
109	RRRRRRRRRRRR
110	RRRRRRRRRRRRRRRR
111	RRRRRRRRRRRRRRRRRRRR
112	RRRRRRRRRRRRRRRRRRRRRRRR
113	YKA
114	KKKKKKKKWKGGGGACYGLPHLFCG
115	YKAKKKKKKKKWK
116	KTPKKAKKPKTPKKAKKP
117	KKAKKPAATRKSSKNPKKPKTVKPKKVAK
118	RGARRYSKMKRRRRRVARRHRRRP
119	TRQARRNRRRRWRERQRGSGSG
120	KRPRGRPKGSKKNWRRRKRRASRRSPRRR
121	KRGRGRPRKQPPKEPSEVPTPKRPRGRPKGSKNK
122	KEKYEKDIAAYRAKGKPAAKKGVVKAEKSKKKK
123	YKAKKKKKKKKKKWK
124	KKKKKKKGGC
125	YRARRRRRRRRWR
126	YRARRRRRRRRRRWR
127	KGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKK
128	KKQLKKQLKKQLKQWK
129	KKSPKKSPKKSPKKSK
130	KLSKLEKKSKLEK
131	KLSKLEKKLSKLEKKSKLEK
132	KSLKKSLKKSLKKSK
133	KIRRRGKNKVAARTCRQRRTDR
134	KIRRRGKNKVAAQNCRKRKLET
135	KRRIRREKNKMAAAKCRNRRRELT

SEQ ID No.	Sequence
136	KDRSNLLERHTR
137	KRPAATKKAGQAKKKL
138	RRRRRREEEE
139	RRRRRREEEEEE
140	RRRRRREEEEEEEE
141	RRRRRRRREEEE
142	RRRRRRRREEEEEE
143	RRRRRRRREEEEEEEE
144	RRRRRRRRRRREEEE
145	RRRRRRRRRRREEEEEE
146	RRRRRRRRRRREEEEEE
147	KLSKLEKK
148	SKLEK
149	KLSKLEKKLSKLEKK
150	PKKKRKVGGGRGDSP
151	LPHKSMPCG
152	GACLQHKSMPCG
153	YGLPHLFCG
154	SERSMNFCG
155	DHYSLYEDLERGTDK
156	ISLPRTSGAQRASTTR
157	EKLQTKYGLPHKVEFCG
158	TRISESQAKPGD
159	LVFFDY
160	WGGNGPTTFDCSGYTKYVFAK
161	INIGTTGWGDHYSLY
162	YDNIHG
163	AGWGKFLVGFGRV
164	SIGYPLP
165	TTHWGFTL
166	HLQIQPYPQISG
167	KLNIVSVNG
168	RGH
169	DNRIRLQAKAA
170	KIKMVISWKG

SEQ ID No.	Sequence
171	LPWYSYLYAVSA
172	WNLPWYYSVSPT
173	WNL
174	PWYYSVSPT
175	SSWESYKSGGGTRL
176	RDWSSQHPGRCNGETHLK
177	SLPTLTL
178	VICTGGDYSFALPVGQWPVMT
179	DKPSYQFGGHNSVDFEEDTLPKV
180	RARRRKRASATQLYQTCKASGTCPPD
181	SGDYSFALPVGQWPWMTG
182	CTGGDYSFALPVGQWPW
183	FYYDYDFFFDYWGQG
184	HLRRLRRRLLREAEG
185	DYYCAAWDDSLNGYSVF
186	YYCLQSMEDPYTFGG
187	YYCARSDGNYGYYYALDYDY
188	AARSPSYYRYDY
189	GPYYAMDYD
190	YYCQQRSSYPYTEGGAYPKAWK
191	YYCQRYDSDWSFGQGTKL
192	YYCARSGYYAMDYWGQGT
193	RVRRGACRGDCLG
194	RVRRGACRYDCLG
195	YYCAKGTHWGFWSGYFDYWGQGT
196	GRENYHGCTTHWGFTLC
197	VQATQSNQHTPRGGGSK
198	DPRAPGS
199	YYCQQRSSYPYTFGG
200	AARSPSYYRYDYGPYYAMDYD
201	GPKLTGILISILSLFVES
202	KYILRWRPKNS
203	IKVAV
204	WTPPRAQITGYRLTVGLTRR
205	AASIKVAVSADR

SEQ ID No.	Sequence
206	KLDAPT
207	NRWHSIYITRFG
208	PHSRN
209	SSFHFDGSGYAM
210	RGDS
211	IAFQRN
212	GRGDSP
213	TWYKIAFQRRK
214	EDGIHEL
215	SLVRNRRVITIQ
216	YRVRVTPKEKTGPMKE
217	LQVQLSR
218	SPPRRARVT
219	RKRLQVQLSIRT
220	ATETTITIS
221	NAPFPKLSWTIQ
222	VSPPRRARVTDATETTITISWRTKTETITGG
223	WTIQTTVDRGLL
224	KPDVRSYTITG
225	DTINNGRDHMILI
226	ANGQTPIQRYIK
227	MILISIGKSQKRM
228	PRARITGYIIKYEKPGSPPREVVPRPRPGV
229	PPFLMLLKGSTR
230	WQPPRARI
231	NQRLASFSNAQQS
232	WQPPRARITGYIIKYEKPG
233	ISNVFVQRMSQSPEVLD
234	YEKPGSPPREVVPRPRPGV
235	KARSFNVNQLLQD
236	KNNQKSEPLIGRKKT
237	KNSFMALYLSKG
238	EILDVPST
239	KNSFMALYLSKGRLVFALG
240	IDAPS

SEQ ID No.	Sequence
241	RDSFVALYLSEGHVIFAGLG
242	VVIDASTAIDAPSNL
243	KPRLQFSLDIQT
244	LDVPS
245	DGQWHSVTVSIK
246	REDV
247	FVLYLGSKNAKK
248	PHSRNRGDSP
249	LAIKNDNLVYVY
250	LWVTVRSQQRGLF
251	AYFSIVKIERVG
252	GTNNWWQSPSIQN
253	DVISLYNFKHIY
254	WVTVTLDLRQVFQ
255	FFDGSSYAVVRD
256	RQVFQVAYIIIKA
257	LHVFYDFGFGFSNG
258	LTRYKITPRRGPPT
259	LKKAQINDAKYREISIIYHN
260	LLEFTSARYIRL
261	RAYFNGQSFIAS
262	YIRLRLQRIRTL
263	SRLRGKNPTKGK
264	RRYYYSIKDISV
265	LHKKGKNSSKPK
266	SINNTAVNQRLT
267	RLKTRSSHGMIF
268	GGFLKYTVSYDI
269	GEKSQFSIRLKT
270	RDQLMTVLANVT
271	TLFLAHGRLVFM
272	ANVTHLLIRANY
273	LVFMFNVGHKKL
274	AGTFALRGDNPQG
275	TLFLAHGRLVFMFNVGHKKL

SEQ ID No.	Sequence
276	VLIKGGRARKHV
277	DFMTLFLAHGRLVFMGNVG
278	LSNIDYLIKAS
279	HKKLKIRSQEKY
280	LQQSRIANISME
281	GAAWKIKGPIYL
282	NLLLLLVKANLK
283	VIRDSNVVQLDV
284	HRDELLLWARKI
285	GLIYYVAHQNQM
286	KRRARDLVHRAE
287	DYATLQLQEGRLHFMFDLG
288	SQFQESVDNITK
289	KKGSYNNIVVHV
290	PGGMREKGRKAR
291	ADNLLFYLGSAK
292	MEMQANLLLDRL
293	GSAKFIDFLAIE
294	LSEIKLLISAR
295	KVSFLWWVGSGV
296	RDFTKATNIRLRFLR
297	SYWYRIEASRTG
298	ISTVMFKFRTFS
299	YFDGTGFAKAVG
300	KQANISIVDIDSN
301	NGQWHKVTAKKI
302	FSTRNESGIILL
303	AKKIKNRLELVV
304	RRQTTQAYYAIF
305	GFPGGLNQFGLTTN
306	YAIFLNKGRLEV
307	NQFGLTTNIRFRG
308	KNRLTIELEVRT
309	IRSLKLTKGTGKP
310	GLLFYMARINHA

SEQ ID No.	Sequence
311	AKALELRGVQPVS
312	VQLRNGFPYFSY
313	GQLFHVAYILIKF
314	HKIKIVRVKQEG
315	NVLSLYNFKTTF
316	DFGTVQLRNGFPFFSYDLG
317	SQRIYQFAKLNYT
318	NIRLRFLRTNTL
319	EVNVTLDLGQVFH
320	GKNTGDHFVLYM
321	GQVFHVAYVLIKF
322	VVSLYNFEQTFML
323	HQQDLGTAGSCLRKFSTMFLF
324	RFDQELRLVSYN
325	HQQDLGTAGSCLRKFSTMFLFCNI
326	RLVSYSGVLFFLK
327	VAEIDGIEL
328	NWRHISYITRFG
329	GIIFFL
330	KRLQVQLRSIRT
331	ASKAIQVFLLGG
332	TWYKIAFQRNRK
333	VLVRVERATVFS
334	QVFQVAYIIIKA
335	TVFSVDQDNMLE
336	GEFYFDLRLKGDK
337	RLRGPQRVFDLH
338	GTPGPQGIA
339	FDLHQNMGSVN
340	GQRDVV
341	LRAHAVDVNG
342	TAGSCLRKFSTM
343	LFSHAVSSNG
344	KGHRGF
345	TAGSCLRKFSTMFLF

SEQ ID No.	Sequence
346	TAGSCLRKFSTMFLFCNI
347	DLGTAGSCLRKFSTM
348	HQQDLGTAGSCLRKFSTM
349	RNIAEIIKDI
350	SIGFRGDGQTC
351	LNRQELFPFG
352	RIQNLLKITNLRIKFVK
353	KKQRFRHRNRKGYRSQ
354	SINNTAVMQRLT
355	FRHRNRKGY
356	RYRVRVTPKEKTGPMKE
357	SETTVKYIFRLHE
358	GHRGPTGRPGKRGKQGQKGDS
359	KAFDITYVRLKF
360	GDLGRPGRKGRPGPP
361	YIGSR
362	RGEFYFDLRLKGDK
363	LAGSCLARFSTM
364	LALFLSNGHFVA
365	ISRCQVCMKKRH
366	PGRWHKVSVRWE
367	TDIPPCPHGWISLWK
368	VRWGMQQIQLVV
369	TAIPSCPEGTVPLYS
370	KMPYVSLELEMR
371	GPAGKDGEAGAQG
372	VLLQANDGAGEF
373	GLPGER
374	DGRWHRVAVIMG
375	LAGSCLPVFSTL
376	APVNVTASVQIQ
377	TAGSCLRRFSTM
378	KQGKALTQRHAK
379	TAGSCLRKF
380	RYVVLPR

SEQ ID No.	Sequence
381	TAGSCL
382	SPYTFIDSLVLMPY
383	TAG
384	PDSGR
385	QQNLGSVNVSTG
386	SRATAQKVSRRS
387	DPGYIGSR
388	GSLSSHLEFVGI
389	VILQQSAADIAR
390	RNRLHLSMLVRP
391	KDISEKVAVYST
392	APMSGRSPSLVLK
393	LGTIPG
394	AFGVLALWGTRV
395	TDIRVTLNRLNTF
396	IENVVTTFAPNR
397	AFSTLEGRPSAY
398	LEAEFHFTHLIM
399	TSAEAYNLLLRT
400	HLIMTFKTFRPA
401	LNRRYEQARNIS
402	KTWGVYRYFAYD
403	SLLSQLNNLLDQ
404	TNLRIKFVKLHT
405	RDIAEIIKDI
406	KRLVTGQR
407	SHAVSS
408	GPGVVVVERQYI
409	ADTPPV
410	NEPKVLKSYYYAI
411	LRAHAVDING
412	YYAISDFAVGGR
413	DSITKYFQMSLE
414	LPFFNDRPWRRAT
415	YTALIIATDN

SEQ ID No.	Sequence
416	FDPELYRSTGHGGH
417	VITVKDINDN
418	TNAVGYSVYDIS
419	GLDRESYPYY
420	APVKFLGNQVLSY
421	MKVSATDADD
422	SFSFRVDRRDTR
423	PQVTRGDVFTMP
424	TWSKVGGHLRPGIVQSG
425	KEAEREVTDLLR
426	RGDV
427	AAEPLKNIGILF
428	FALWDAIIGEL
429	VGVAPG
430	LWPLLAVLAAVA
431	PGVGV
432	VFDNFVLK
433	TSIKIRGTYSER
434	TTSWSQCSKS
435	DPETGV
436	KRSR
437	QGADTPPVGV
438	SVVYGLR
439	PLDREAIAKY
440	DGRGDSVAYG
441	HAVDI
442	LALERKDHSG
443	DQNDN
444	YSMKKTTMKIIPFNRLTIG
445	QDPELPDKNM
446	RGDF
447	LVVQAADLQG
448	GVYYQGGTYSKAS
449	NDDGGQFVVT
450	TAGSCLRKFSCL

SEQ ID No.	Sequence
451	YILHVAVTN
452	CNYYSNSYSFWLASLNPER
453	TYRIWRDTAN
454	TGLSCLQRFTTM
455	GFTCECSIGFRGDGQTCYGIVFWSEV
456	HHLGGAKQAGDV
457	SCLPGFSGDGRACRDVDECGH
458	MAPRPSLAKKQRFRHRNRKGYRSQRGHSRG
459	KKQKFRHRNRKGYRSQ
460	KKQKFKHRNRKGYRS
461	KKQKFRRRNRKGYRSH
462	TAIPPCPHGWISLWK
463	KKQKSRHRSRKRYRS
464	KKQKSRRRSRKGYRS
465	ISRCTVC
466	ISRCQVCMKRRH
467	VSRCTVC
468	TDIPPCPQGWISLWK
469	TVKAGELEKIISRCQVMKKRH
470	TDIPSCPHGWISLWK
471	TDIPPCPAGWISLWK
472	TEIPPCPQGWISLWK
473	TDVPPCPQGWISLWK
474	RLVSYNGILFFLK
475	RLVSYSGVIFFLK
476	RLVSYNGILFFL
477	RLVSYSGIIFFLK
478	RFEQELRLVSYSGVLFFLKQ
479	RLVSYNGIIFFLK
480	DPAFKIEDPYSPRIQNLLKITNLRIKFVKL
481	TKRFEQELRLVSYSGVLFFL
482	GGRLKYSVAF
483	GGFLRYTVSYDI
484	GGFLKYTVSYDV
485	LGNKLTAFGGFLKYTVSYDIPV

SEQ ID No.	Sequence
486	GGYLKYTVSYDI
487	GEIFFDMRLKGDK
488	GEIYFDLRLKGDK
489	GEIYLDMRLKGDK
490	IGQPGAKGEPGEFYFDLRLKGDKGDPGFPG
491	GEVFFDMRLKGDK
492	LAGSCLPIFSTL
493	AHNQDLGLAGSCLARFSTMPFLYCNPGDIC
494	QEKAHNQDLGLAGSCLPVFSTLPFAYCNIH
495	LAGSCLPVFSTM
496	GNKRAHGQDLGTAGSCLRRFSTMPFMFCNI
497	RAHGQDLGTAGSCLRRFSTMP
498	RKRLQVQLNIRT
499	HLVLPLQQSDVRKRLQVQLSIRTFASSGLI
500	RKRLSVQLRIRT
501	DLGTAGSCLRRFSTM
502	RNIAEIIKDI
503	TAGSCLRKFSTMRRRRRRRRRRR
504	FTLTGLLGTLVTMGLLT
505	APYKAWK
506	STSKTNRGDDSNWSKRVTNNKPS
507	STSKRKRGDDSNWSKRVTKKKPS
508	STSKRKRGDDSNWSKRVSKKKPS
509	STSKRKRGDDANWSKRVTKKKPS
510	PLAGSKRKRADEVAWSKRGTKKKPER
511	PLAGSKRKRADEVAWSKRGTKKKPERTSAARAGPSRRIR
512	STSKRKRGDDANWSKRTTKKKPSS
513	STSKRKRGDDANWSKRTTKKKPSSAGLKRAGSKADRPSL
514	PTTAGKRKRSDDAAWSKRARPKAGRT
515	PTTAGKRKRSDDAAWSKRARPKAGRTSAARPGTSVRRIR
516	SSSLGKRKRSDEGAWSKGKSKKKAMR
517	SSSLGKRKRSDEGAWSKGKSKKKAMRGSSSRRPGPVRGP
518	PTTAGKRKRTDDAAWSKRARPKAGR
519	PTTAGKRKRTDDAAWSKRARPKAGRTSAARPGTAVRRVR
520	PATAGKRKRSDDAAWSKRARPKAGRTSAAR

SEQ ID No.	Sequence
521	PATAGKRKRSDDAAWSKRARPKAGRTSAARPGTSVRRIR
522	SSSLGKRKRSNGGDWSKRSAKKKPA
523	SSSLGKRKRSNGGDWSKRSAKKKPAGTPSRRAGPGRGPR
524	SSSLGKRKRSDEGAWSKGKSKKKAMR
525	SSSLGKRKRSDEGAWSKGKSKKKAMRGSSSRRPGPVRGP
526	STSKRKRGDDANWNKRPTKKKPSS
527	STSKRKRGDDANWNKRPTKKKPSSAGLKKAGSKAERPSL
528	SGALKRKRSDEVAWSRRRPVKKPV
529	SGALKRKRSDEVAWSRRRPVKKPVRRAPPPRAGPSVRRG
530	SGALKRKRSDEVAWSRRKPAKKPAR
531	SGALKRKRSDEVAWSRRKPAKKPARQPPPPRAGPSVRRG
532	AGALKRKRSDEVAWSRRKPAKKPAR
533	AGALKRKRSDEVAWSRRKPAKKPARAPPPRAGPSVRRGL
534	STSKRKRGDDSNWSKRVTKKKPSSAGLKRAGSKADRPSLQI QTLQHAGTTMITVPSGGVCDLINTYARGSDEGNRHTSETLTY KIAIDYHFVADAAACRYSNTGTGVMWLVYDTTPGGQAPTPQ TIFSYPDTLKAWPATWKVSRELCHRFVVKRRWLFNMETDGR IGSDIPPSNASWKPCKRNIYFHKFTSGLGVRTQWKNVTDGGV GAIQRGALYMVIAPGNGLTFTAHGQTRLYFKSVGNQ
535	DPQNALYYQPRVPTAAPTSGGVPWSRVGEVAILSFVALICFY LLYLWVLRDLILVLKARQGRSTEELIFGGQAVDRSNPIPNIPA PPSQGNPGPFVPGTG
536	GSQLVPPPSAFNYIESQRDEFQLSHDLTEIVLQFPSTASQITAR LSRSCMKIDHCVIEYRQQVPINASGTVIVEIHDKRMTDNESLQ ASWTFPIRCNIDLHYFSSSFFSLKDPIPWKLYYRVSDSNVHQM THFAKFKGKLKLSSAKHSVDIPFRAPTVKILAKQFSEKDIDFW HVGYGKWERRLVKSASSSRFGLRGPIEINPGESWATKSAIVTP NRNADLDIEEELLPYRELNRLGTNILDPGESASIVGIQRSQSNI TMSMSQLNELVRSTVHECIKTSCIPSTPKSLS
537	RTGVKRSYGAARGDDRRRPNVV
538	SYVKTVPNRTRTYIKLRVR
539	MYSTSNRRGRSQTQRGSHVRRTGVKRSYGAARGDDRRRPN VVSKTQVEPRMTIQRVQENQFGPEFVLSQNSALSTFVTYPSY VKTVPNRTRTYIKLKRVRFKGTLKIERGQGDTIMDGPSSNIEG VFSMVIVVDRKPHVSQSGRLHTFDELFGARIHCHGNLSVVPA LKDRYYIRHVTKRVVSLEKDTLLIDLHGTTQLSNKRYNCWA SFSDLERDCNGVYGNITKNALLVYYCWLSDAQSKASTYVSF ELDYLG
540	RRRRRRRRRRRRVDYGKWERKPIRCASMSR

SEQ ID No.	Sequence
541	RRRRRRRRRRRRGKWERKPIRCAS
542	KKKKKKKKKKKKKKKKGKWERKPIRCAS
543	RRRRRRRRRRRRVDFSHVDYGKWERKPIRCASMSRLGLRG
544	GVKRSYGAARGDDRRRPNVVAPYKAWRRRRRRRRRRR
545	KSVPNRTRTYIKLKRLRFKGAPYKAWRRRRRRRRRRR
546	RTGVKRSYGAARGDDRRRPNVVRRRRRRRRRRR$_2$
547	SYVKTVPNRTRTYIKGGGGGRRRRRRRRRRR$_2$
548	VDIPFRAPTIKILSKQFTEDDIDFWHVGYGKWERKLVRPASLS GRRGLRR
549	IDFWHVGYGKWERKLVRPASLSGRRGLRR
550	IDFWSVEKGETRRRLLNPTPHAHSPRPIAHR
551	IDFSHVGYGKWERKMIRSASISRLGLHN
552	VDFSHVGYGKWERKLIRSASTVKYGLPS
553	IDFSHVDYGKVERKLVKCESSSRLGLHS
554	IDFWSVGRKAQQRKLVQGPSLIGSRSMRY
555	IDFWSVGSKPQTRRLVDGSRLIGHSSRSLRV
556	IDFWSVERGETRRRLLNPTPSAGSNRALSKR
557	VDFWSVGKPKPIRRLIQNDPGTDYDTGPKYR
558	VDFWSVEKPKPIRRLLNPGPNQGPYPNTGHR
559	VDFSHVDYGKWERKLIRSASTSRYGLRS
560	VDFSHVDYGKWERKTLRSRSLSRIGLTG
561	IDFWHVGYGKWERRLVKSASSSRFGIRG
562	VDFFHVDYGRWERKHIRCASMSRVGLRG
563	GTFQHVDYGKWERKPIRCQSMSRVGYRR
564	VGYGKWERKLVRPASLS
565	VEKGETRRRLLNPTPHA
566	VGYGKWERKLIRSASTV
567	VEKPKPIRRLLNPGPNQ
568	VDYGKWERKLIRSASTS
569	VDYGKWERKTLRSRSLS
570	VGYGKWERRLVKSASSS
571	VDYGRWERKHIRCASMS
572	VERPKPIRRLLTPTPGC
573	PFRAPTIKILSKQFTEDDIDFWHVGYGKWERKLVRPASLSGR RGLRR

SEQ ID No.	Sequence
574	PFRAPTVKILSKQFTDKDIDFSHVGYGKWERKMIRSASISRLG L
575	DIAFRAPTVKILSKQFTDRDVDFSHVGYGKWERKLIRSASTV KYGL
576	DIRFKPPTINILSKDYTADCVDFWSVEKPKPIRRLLNPGPNQGP YPNTG
577	DIPFRAPTVKIHSKQFSHRDVDFSHVDYGKWERKTLRSRSLS RIGL
578	DIPFRAPTVKILAKQFSEKDIDFWHVGYGKWERRLVKSASSS RFGI
579	DIPFRAPTVKILSKQFTDKDVDFFHVDYGRWERKHIRCASMS RVGL
580	DIKYKPPTIKILSKDYTADCVDFWSVERPKPIRRLLTPTPGCG
581	ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVK KPHRYR PGTVA
582	SGRGKGGKG LGKGGAKRHRKVLRDNIQGITKPAI
583	GRKKRRQRRR

METHODS OF USING LIPID COMPOSITIONS

[0191] Use of these compositions in transfection can be carried out by methods that are known in the art. For example, the compositions described herein can be used to transfect cells *in vitro* or *ex vivo*.

[0192] For *in vitro* delivery, WO07/130073, at pages 54-60 describes "before" and "after" protocols for transfection where the components of a transfection complex are mixed in differing orders prior to addition to a cell culture. Typically, a liposomal preparation of the lipid, with or without colipid is prepared, and is then mixed with a payload/macromolecule, such as a nucleic acid molecule, to form a transfection complex. The complex is then added to a cell culture and transfection is monitored using well known methods. Additional components such as cell surface ligands, fusion agents, nuclear localization agents and the like may be added to the nucleic acid prior to admixture with the liposome, or may be added to the liposome prior to addition of nucleic acid. In some embodiments, the contacting of the cell occurs *in vitro*. In some embodiments, the contacting of the cell occurs *ex vivo*. In some embodiments, the contacting of the cell occurs *in vivo*.

[0193] Cells which can be transfected according to these methods include, but are not limited to, virtually any eukaryotic cell including primary cells, cells in culture, a passaged cell culture or a cell line, and cells in cultured tissue. Suitable cells include human cell lines and animal cell lines. The cell may be a fibroblast, an immune cell (e.g., a T cell, and NK cell) a pluripotent cell (e.g., a pluripotent stem cell, an iPSC, a neural stem cell, a mesenchymal stem cell, a progenitor cell, or the like). The cells can be attached cells or cells in suspension (suspension cells). The cells can also be an organoid or a spheroid. In certain illustrative aspects, the cells are suspension CHO-S cells and suspension 293-F cells. Other cells that may be used include, without limitation, 293, 293-S, CHO, CHO K-1, Cos, 3T3, Hela, primary fibroblasts, A549, Be2C, SW480, CHOK1, Griptite 293, HepG2, Jurkat, LNCap, MCF-7, NIH-3T3, PC12, C6, Caco-2, COS-7, HL60, HT-1080, IMR-90, K-562, SK-BR3, PHP1, HUVEC, MJ90, NHFF, NDFF and primary neurons. In some embodiments, the cell is a mammalian cell.

[0194] For *in vivo* administration, the lipid nanoparticle formulations are preferably administered parenterally, *i.e.*, intraarticularly, intravenously, intraperitoneally, subcutaneously, or intramuscularly. In particular embodiments, the pharmaceutical compositions are administered intravenously or intraperitoneally by a bolus injection. For one example, see Stadler, et al., U.S. Pat. No. 5,286,634, which is incorporated herein by reference. Intracellular nucleic acid delivery has also been discussed in Straubringer, *et al.*, *Methods in Enzymology*, Academic Press, New York. 101:512-527 (1983); Mannino *et al.*, *Biotechniques* 6:682-690 (1988); Nicolau *et al.*, *Crit. Rev. Ther. Drug Carrier Syst.* 6:239-271 (1989), and Behr, *Acc. Chem. Res.* 26:274-278 (1993). Still other methods of administering lipid-based therapeutics are described in, for example, Rahman *et al.*, U.S. Pat. No. 3,993,754; Sears, U.S. Pat. No. 4,145,410; Papahadjopoulos *et al.*, U.S. Pat. No. 4,235,871; Schneider, U.S. Pat. No. 4,224,179; Lenk *et al.*, U.S. Pat. No. 4,522,803; and Fountain *et al.*, U.S. Pat. No. 4,588,578. In some embodiments, the administration is systemic.

[0195] In some embodiments, the administration is selected from: subcutaneous administration, intramuscular administration, intranasal administration, intra-tumoral administration, administration to the brain, administration to the spinal cord, administration to the eye, administration to the lymph node of a subject, and any combination thereof.

[0196] In another embodiment is a method for producing a protein which includes contacting a cell with a lipid-nucleic acid complex as described above, where the nucleic acid encodes the protein. In some embodiments, protein production is in vitro, e.g., bioproduction. In this type of application, cells are incubated to produce the protein and the protein is collected. Cells which can be used for protein production are described above. In addition, any composition which includes a lipid of Formula (I) or (II) can be used for transfection of cells. Such compositions are further discussed herein, and include, but are not limited to compositions comprising lipids of Formula (I) or (II), a co-lipid and an optional transfection enhancing agent such as a fusogenic peptide or protein. For example, the methods provided herein include methods of producing therapeutic or prophylactic proteins, either *in vivo* or *in vitro*. By way of example, the compositions provided herein can be used to deliver mRNA encoding immunogens (e.g, for vaccines), mRNA encoding therapeutic proteins (e.g, growth factors, enzymes, cytokines, or the like), or the like.

[0197] In another embodiment is a method for inhibiting production of a protein in a cell, comprising contacting the cell with a lipid-nucleic acid complex as described above, where the nucleic acid is a double stranded RNA molecule, such as an RNAi or siRNA molecule designed to inhibit expression of the protein. Methods of designing such RNA molecules are well known in the art. Lipids of Formula (I) are particularly suitable for deliver of RNAi molecules in this fashion. The cells are incubated and the phenotypic consequence of inhibiting production of the selected protein is observed.

[0198] An aspect of the present application relates to a method of delivering a payload to a cell. The method includes providing a composition described herein; providing a cell; and contacting the cell with the composition.

[0199] Another aspect of the present application relates to a method for delivering a composition to a subject. The method includes administering the composition described herein to the subject.

METHODS OF MANUFACTURE AND METHODS OF USE OF LIPID FORMULATIONS

[0200] The lipids described above may be formulated by various methods to be used in transfection. One of the simplest methods for formulation is reverse evaporation, as described in

U.S. Pat. No. 9,259,475, which is hereby incorporated by reference in its entirety. Other methods for formulation that can be used are sonication and microfluidization. Advantageously, the lipids are formulated as lipid nanoparticles using microfluidic mixing as described, for example, in Roces *et al., Pharmaceutics*, 12:1095 (2020). Suitable microfluidic mixing devices are commercially available from, for example, Precision Nanosystems (Vancouver, BC). Typically, microfluidic mixing combines two fluid streams, one containing the nucleic acid(s) and one containing the lipid of Formula (I) and other components, such as the peptide, ligand and other lipid components as described below.

[0201] For lipid nanoparticle compositions including an RNA, solutions of the RNA at concentrations of 0.1 mg/ml in deionized water are diluted in 50 mM sodium citrate buffer at a pH between 3 and 4 to form a stock solution. Nanoparticle compositions can be processed by dialysis to remove ethanol and achieve buffer exchange. Formulations may be dialyzed against phosphate buffered saline (PBS), pH 7.4, using a desired molecular weight cutoff, *e.g.* 10 kD. The resulting nanoparticle suspension may be filtered through a 0.2 μm sterile filters (Sarstedt, Numbrecht, Germany) into glass vials and sealed.

[0202] Methods of determining particle size in nanoparticles formulations are well-known in the art. For example, a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) can be used to determine the particle size, the polydispersity index (PDI) and the zeta potential of the nanoparticle compositions. UV-visible spectroscopy can be used to determine the concentration of nucleic acid (*e.g.*, mRNA) in nanoparticle compositions. A quantity of the composition is diluted in a suitable solvent and the absorbance spectrum of the solution is recorded, for example, between 230 nm and 330 nm on a spectrophotometer. The concentration of therapeutic and/or prophylactic in the nanoparticle composition can be calculated based on the extinction coefficient of the therapeutic and/or prophylactic used in the composition and on the difference between the absorbance at a wavelength of, for example, 260 nm and the baseline value at a wavelength of, for example, 330 nm.

[0203] For nanoparticle compositions including an RNA, a QUANT-IT™ RIBOGREEN® RNA assay (Invitrogen Corporation, Carlsbad, CA) can be used to evaluate the encapsulation of an RNA by the nanoparticle composition using methods provided by the manufacturer. The fluorescence intensity generated after addition of the RIBOGREEN reagent can be measured

using a fluorescence plate reader at an excitation wavelength of, for example, about 480 nm and an emission wavelength of, for example, about 520 nm. The fluorescence values of the reagent blank are subtracted from that of each of the samples and the percentage of free RNA is determined by dividing the fluorescence intensity of the intact sample (without addition of Triton X-100) by the fluorescence value of the disrupted sample (caused by the addition of Triton X-100).

REAGENT KITS

[0204] The present invention also provides packaging and kits comprising pharmaceutical compositions for use in the methods of the present invention. In embodiments, a lipid composition in the kit is suitable for delivery (e.g., local injection) to a subject.

[0205] The kit can comprise one or more containers selected from the group consisting of a bottle, a vial, an ampoule, a blister pack, and a syringe. The kit can further include one or more of instructions for use in treating and/or preventing a disease, condition or disorder of the present invention, one or more syringes, one or more applicators, or a sterile solution suitable for reconstituting a pharmaceutical composition of the present invention.

[0206] In some examples, the kit comprising a lipid composition provides that the ionizable lipid and neutral lipid are in a separate container from the peptide. In some examples, the kit comprising a lipid composition provides that the ionizable lipid and neutral lipid are in the same container as the peptide.

[0207] Components of the transfection compositions described above can be provided in a reagent kit. The kits contain the lipid of Formula (I) above, together with additional components, such as a neutral lipid, a cationic lipid, cell surface ligands, fusion agents, and/or nuclear localization agents and the like. The kit components may be separate or may be premixed in any manner. For example, the lipid of formula (I) may be admixed with one or more neutral lipid. Additional components may also be present in the same container or may be present in one or more separate containers. The kits typically include vessels, such as vials and/or tubes, which are packaged together, for example in a cardboard box. The kits can be shipped from a supplier to a customer. For example, in one example provided herein is a kit that includes a vial that includes a liposomal formulation as described above and, optionally, a transfection agent and a

transfection enhancing peptide. The kit can also include, for example, a separate vessel that includes a transfection enhancing agent, such as a transfection enhancing peptide, for example Plus Reagent™ (Invitrogen Corp., Carlsbad, CA). The kit can also include in separate containers, cells, cell culture medium, and a reporter nucleic acid sequence, such as a plasmid that expresses a reporter gene. In certain examples, the culture medium can be reduced-serum medium and/or protein expression medium.

[0208] Also provided are kits containing a compound of Formula (I) or (Ia) and additional reagents such as a cationic lipid, a neutral lipid, an amphipathic peptide, an amphipathic peptide comprising a polycationic nucleic acid binding moiety, a cell surface ligand, a cell surface ligand comprising a polycationic nucleic acid binding moiety, a fusion agent, a fusion agent comprising a polycationic nucleic acid binding moiety, a nuclear localization peptide or protein, and a nuclear localization peptide or protein comprising a polycationic nucleic acid binding moiety. The kits may contain one, some, or all of these additional reagents, in any possible combination. Advantageously, the additional reagents include a cationic lipid, an amphipathic peptide and a cell surface ligand that contains a polycationic nucleic acid binding moiety. When the cell surface ligand is a peptide or protein, the polycationic nucleic acid binding moiety is a polybasic amino acid sequence.

[0209] In one embodiment, a kit comprises individual portions of, or a mixture of, cationic lipid, such as a lipid of Formula (I) or (Ia) and peptide, protein or fragment thereof or modified peptide, protein or fragment thereof. In another embodiment, a kit comprises individual portions of, or a mixture of, polycationic polymers and peptide, protein or fragments thereof or modified peptide, protein or fragments thereof. Cationic lipid transfection kits can optionally include neutral lipid as well as other transfection-enhancing agents or other additives, and the relative amounts of components in the kit may be adjusted to facilitate preparation of transfection compositions. Kit components can include appropriate medium or solvents for other kit components.

[0210] An aspect of the present application relates to a kit including one or more compounds described herein and one or more of a structural lipid, an ionizable lipid, and a stabilizing agent. In some embodiments, the kit includes one or more compounds described herein, one or more of a structural lipid, one or more stabilizing agent and optionally a payload. In some embodiments,

the kit includes one or more compounds described herein, one or more of a structural lipid, one or more stabilizing agent, one or more fusion agent, and optionally a payload.

[0211] Payloads that can be delivered by the methods of this invention include nucleic acids, proteins, ribonucloeproteins, and the like, including DNA and RNA (including RNAi/siRNA) of any size from any source comprising natural bases or non-natural bases, and include those encoding and capable of expressing therapeutic or otherwise useful proteins in cells, those which inhibit undesired expression of nucleic acids in cells, those which inhibit undesired enzymatic activity or activate desired enzymes, those which catalyze reactions (ribozymes), and those which function in diagnostic assays (e.g., diagnostic nucleic acids). Therapeutic nucleic acids include those nucleic acids that encode or can express therapeutically useful proteins, peptides or polypeptides in cells, those which inhibit undesired expression of nucleic acids in cells, and those which inhibit undesired enzymatic activity or activate desired enzymes in cells. In certain embodiments, the payload comprises an RNA molecule. The compositions can be used to deliver RNA payloads such as mRNA, siRNA, shRNA, miRNA, self-replicating RNA (srRNA), self-amplifying RNA, stRNA, sgRNA, or combinations thereof. In some embodiments, the RNA molecule comprises more than one RNA molecule, e.g., more than one mRNA. The compositions and methods provided herein can also be readily adapted in view of the disclosure herein to introduce biologically active macromolecules other than nucleic acids including, among others, polyamines, polyamine acids, polypeptides and proteins into eukaryotic cells. Other materials useful, for example as therapeutic agents, diagnostic materials, research reagents, which can be bound to the peptides and modified peptides and introduced into eukaryotic cells by the methods of this invention. Yet other payloads include small molecules, nutrients, and the like.

[0212] The compositions provided herein can be delivered to cells via in vivo administration. For in vivo administration, the pharmaceutical compositions are preferably administered parenterally (e.g., intraarticularly, intravenously, intraperitoneally, subcutaneously, intrathecally, intradermally, intratracheally, intraosseous, intramuscularly or intratumorally). In particular embodiments, the pharmaceutical compositions are administered intravenously, intrathecally, or intraperitoneally by a bolus injection. Other routes of administration include topical (skin, eyes, mucus membranes), oral, pulmonary, intranasal, sublingual, rectal, and vaginal administration.

[0213] Typical applications include using well known procedures to provide intracellular delivery of siRNA to knock down or silence specific cellular targets in vitro and in vivo. Alternatively, applications include delivery of DNA or mRNA sequences that code for therapeutically useful polypeptides. In this manner, therapy is provided for genetic diseases by supplying deficient or absent gene products. Methods of the present invention may be practiced in vitro, ex vivo, or in vivo. For example, the compositions of the present invention can also be used for delivery of payloads to cells in vivo, using methods which are known to those of skill in the art. In another example, the compositions of the invention can be used for delivery of a payload to a sample of patient cells that are ex vivo, then are returned to the patient.

[0214] For in vivo administration, the pharmaceutical compositions are preferably administered parenterally (e.g., intraarticularly, intravenously, intraperitoneally, subcutaneously, intrathecally, intradermally, intratracheally, intraosseous, intramuscularly or intratumorally). In particular embodiments, the compositions provided herein are administered intravenously, intrathecally, or intraperitoneally by a bolus injection. Other routes of administration include topical (skin, eyes, mucus membranes), oral, pulmonary, intranasal, sublingual, rectal, and vaginal.

[0215] For ex vivo applications, the compositions provided herein are preferably administered to biological samples that have been removed from the organism, then the cells are washed and restored to the organism. The organism may be a mammal, and in particular may be a mammal (e.g., a primate), such as a human. This process is used for cell reprogramming, genetic restoration, immunotherapy, for example.

PHARMACEUTICAL COMPOSITIONS

[0216] According to the present disclosure, nanoparticle compositions can be formulated in whole or in part as pharmaceutical compositions. Pharmaceutical compositions can include one or more nanoparticle compositions. For example, a pharmaceutical composition can include one or more nanoparticle compositions including one or more different therapeutic and/or prophylactic agents. Pharmaceutical compositions can further include one or more pharmaceutically acceptable excipients or accessory ingredients such as those described herein. General guidelines for the formulation and manufacture of pharmaceutical compositions and agents are available, for example, in Remington's The Science and Practice of Pharmacy, 21st

Edition, A. R. Gennaro; Lippincott, Williams &Wilkins, Baltimore, Md., 2006. Conventional excipients and accessory ingredients can be used in any pharmaceutical composition, except insofar as any conventional excipient or accessory ingredient can be incompatible with one or more components of a nanoparticle composition.

OTHER EMBODIMENTS

[0217] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

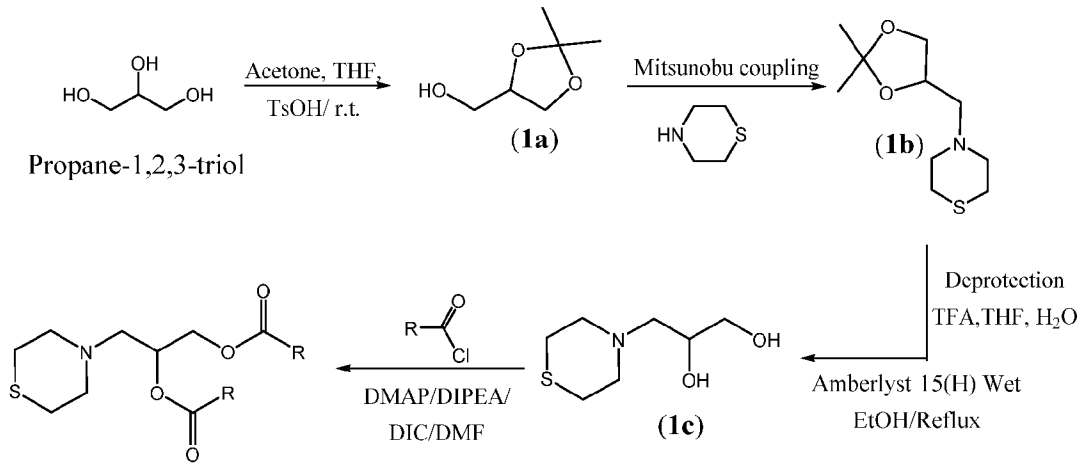
[0218] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All references, *e.g.*, U.S. patents, U.S. patent application publications, PCT patent applications designating the U.S., published foreign patents and patent applications cited herein are incorporated herein by reference in their entireties. GenBank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0219] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

EXAMPLES

EXAMPLE 1: Synthesis of Lipids

[0220] Exemplary lipids were prepared according to the reaction schemes below:



(**36**) R = Myristoyl
(**38**) R = Oleoyl;
(**11**) R = Linoleoyl;
(**37**) R = Palmitoyl

Scheme 5

[0221] Synthesis of (2,2-Dimethyl-1,3-dioxolan-4-yl) methanol (1a).

Glycerol (30g, 0.325 mol.), acetone (46 mL) and para-toluenesulfonic acid monohydrate (1.27g, 3.5% mol.) and chloroform (200 mL) was refluxed for 24 hours in a setup equipped with a Dean Stark trap. Periodically, an aliquot of chloroform is removed from the trap to prevent distilling water from returning to the flask. The reaction mixture was allowed to cool down to room temperature and sodium carbonate (1.3g, 3.6% mol.) was added followed by an additional 30 minutes stirring. The mixture was then filtered, and the solvent evaporated under reduced pressure. The product was purified by distillation under reduced pressure (11 mbar) and collected the fraction distilling between 70-74 °C to yield a pure colorless oil (**1a**) (26.4g, 61.3%). TLC analysis on silica plate, eluent CH_2Cl_2-MeOH 9:1 confirmed formation of the desired product.

[0222] Synthesis of 4-((2,2-dimethyl-1,3-dioxolan-4-yl) methyl) thiomorpholine (1b).

Triphenyl phosphine (2.183g, 8.32 mmol, 1.1 eq.) was dissolved in 30 mL of dry THF under inert atmosphere and the temperature was lowered to -78 °C using a methanol/dry ice bath. Diethyl azo dicarboxylate (DEAD) (1.449g, 1.1 eq.) was added dropwise and the mixture stirred for 30 minutes. Then the protected glycerol (**1a**) (1.0, 7.56 mmol, 1.1 eq.) was added dropwise. The mixture was allowed to stir for 60 minutes at -78 °C. Finally, thiomorpholine (0.78g, 1 eq.) was added dropwise as solution in dry THF (5mL). The reaction mixture was stirred and allowed to warm up slowly without removing the bath. Stirring at room temperature continued overnight

(about 17h) and then the solvent was evaporated and the crude product (5.95g) was loaded on a 50g silica flash chromatography column with a gradient of dichloromethane/acetone gradient. The desired product (**1b**) was obtained as a colorless oil (0.99, 60%). m/z 218.1.

[0223] Synthesis of 3-thiomorpholinopropane-1,2-diol (1c).

The 1,3-dioxolan thiomorpholine (**1b**) (1.0g, 4.6mmol) was dissolved in 95% aqueous Ethanol (15 mL). Amberlyst 15(H) (0.25g) was added and mixture refluxed under vigorous magnetic stirring. The reaction progress was monitored using TLC. The reaction was completed after 5h, after which the resulting 3-thiomorpholine-1,2-diol (**1c**)(0.885g) was using in the next step without further purification.

[0224] General procedure for synthesis of Compounds 36, 38, 11, and 37

[0225] Synthesis of 3-thiomorpholinepropane-1,2-diyl di-myristate (36):

3-thiomorpholine-1,2-diol (**1c**) (0.44g, 2.37 mmol.) was dissolved in dry methylene chloride (20mL). Di-isopropyl ethyl amine (DIPEA) (2.82 mL, 7 eq.) and myristoyl chloride (2.34g, 4 eq.) were added and the mixture stirred at room temperature and inert atmosphere overnight (about 17h). The reaction mixture was then diluted with dichloromethane, washed twice with water (30mL), dried over anhydrous sodium sulfate, filtered off, and the solvent evaporated under reduced pressure to afford 3.25g of crude product. The crude product was taken up in a mixture of hexane-dichloromethane 9:1 (20mL) resulting in the precipitation of a white solid which was filtered off. The remaining oily mixture (1.70g) was loaded over a pre-packed silica flash chromatography column (50g) and separated using a gradient of hexane-dichloromethane to afford the expected product **36** (0.288g). MS: $(M + CH_3OH)H^+$, m/z 631.5.

[0226] Synthesis of 3-thiomopholinepropane-1,2-diyl di-oleate (38)

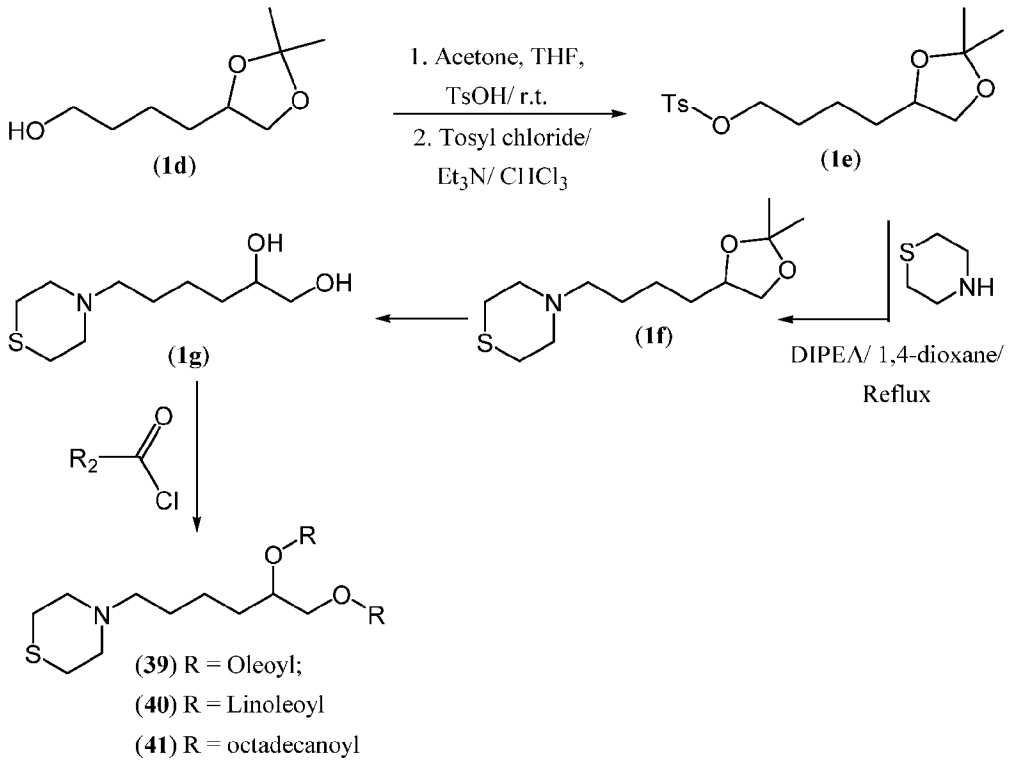
Compound **38** was obtained by treating 3-thiomorpholine-1,2-diol (**1c**) with oleoyl chloride to yield 0.315g. MS: $M^{+\cdot}$ m/z 705.5.

[0227] Synthesis of 3-thiomopholinepropane-1,2-diyl di-linoleate (11)

Compound **11** was obtained by treating 3-thiomorpholine-1,2-diol (**1c**) with linoleoyl chloride to yield 0.484g. MS: $(M + 2MeOH.H_2O)$ m/z 787.6 and $(M + 3MeOH)H^+$ m/z 801.6.

[0228] Synthesis of 3-thiomopholinepropane-1,2-diyl di-palmitate (37)

Compound **37** was obtained by treating 3-thiomorpholine-1,2-diol (**1c**) with palmitoyl chloride to yield 0.86 g. MS: $(M + CH_3OH)H^+$ m/z 687.5.



Scheme 6

[0229] Synthesis of 4-(2,2-dimethyl-1,3-dioxolan-4-yl) butan-1-ol (1d)

A mixture of 1,2,6-hexanetriol (6.5g, 48.8 mmol.), acetone 10mL, para-toluenesulfonic acid (0.32g, 3.5% mol.) and chloroform (20mL) was refluxed for 24 hours in a setup equipped with a Dean Stark trap. Periodically, an aliquot of chloroform is removed from the trap to prevent distilling water from returning to the flask. The reaction mixture was then allowed to cool down to room temperature and sodium carbonate (0.18g, 3.6% mol.) was added followed by additional 30 minutes stirring. The resulting mixture was then filtered, and solvent evaporated under reduced pressure. The product was purified by flash chromatography on silica to yield 2.84g.

[0230] Synthesis of 4-(2,2-dimethyl-1,3-dioxolan-4-yl) butyl-4-*p*-toluenesulfonate (1e)

A mixture of the dioxolane alcohol (**1d**) derivative (6) (0.78g, 4.47 mmol.), triethyl amine (0.81 mL, 1.5 eq.) and p-toluenesulfonyl chloride (0.94g, 1.1 eq) in anhydrous chloroform (30mL) was heated to 60 °C for 8h. TLC monitoring showed that reaction was not completed.

Additional para-toluenesulfonyl chloride (0.34g, 0.5eq.) and triethyl amine (0.32mL, 0.5eq.) were added and solution heated overnight. TLC analysis showed the presence of a small amount of starting material. Another batch of 0.5 eq. para-toluenesulfonyl chloride and triethyl amine were added and additional 6h heating was applied, after which TLC analysis showed completion. At room temperature, the reaction mixture was then diluted with 30 mL of chloroform and the organic layer washed three time with water (3 x 25mL). The organic layer was dried over sodium sulfate, filtered, and solvent evaporated under reduced pressure. The product 1.74g was purified a silica flash chromatography column eluting with a gradient of hexane-ethyl acetate and 1.06g (72.6%) of pure product (**1e**) was obtained. LC/MS: 24.47 min, MH^+ m/z 329.1.

[0231] Synthesis of 4-(4-(2,2-dimethyl-1,3-dioxolan-4-yl) butyl) thiomorpholine (1f)

A mixture of the tosylate derivative (**1e**) (6) (0.96g, 2.92 mmol.), thiomorpholine (0.60g, 2.0 eq.) and di-isopropyl ethyl amine (1,13g, 1.49 mL, 3.0 eq.) was refluxed in 1,4-dioxane (5 mL) for 2.5h. TLC indicated completion of the reaction and then the solvent was evaporated and the crude product (1.62g) was loaded on silica flash chromatography. Separation was performed using a gradient of dichloromethane-acetone. The product (**1f**) was characterized with LC/MS 5.68 min., MH^+ m/z 260.1.

[0232] Synthesis of 6-thiomorpholinohexane-1,2-diol (1g)

The dioxolane derivative (**1f**) (1.27g, 4.89 mmol) was dissolved in 95% aqueous ethanol and amberlyst 15(H) wet (0.6g) was added. The mixture was refluxed under an inert atmosphere with vigorous magnetic stirring for 12h. At room temperature, the suspension was filtered over a pad of celite and solvent evaporated under high vacuum. The product (**1g**) was used in the next step without any further purification.

[0233] Synthesis of 3-thiomorpholinopropane-1,2-diyl (9Z, 9'Z)-bis-oleate (39)

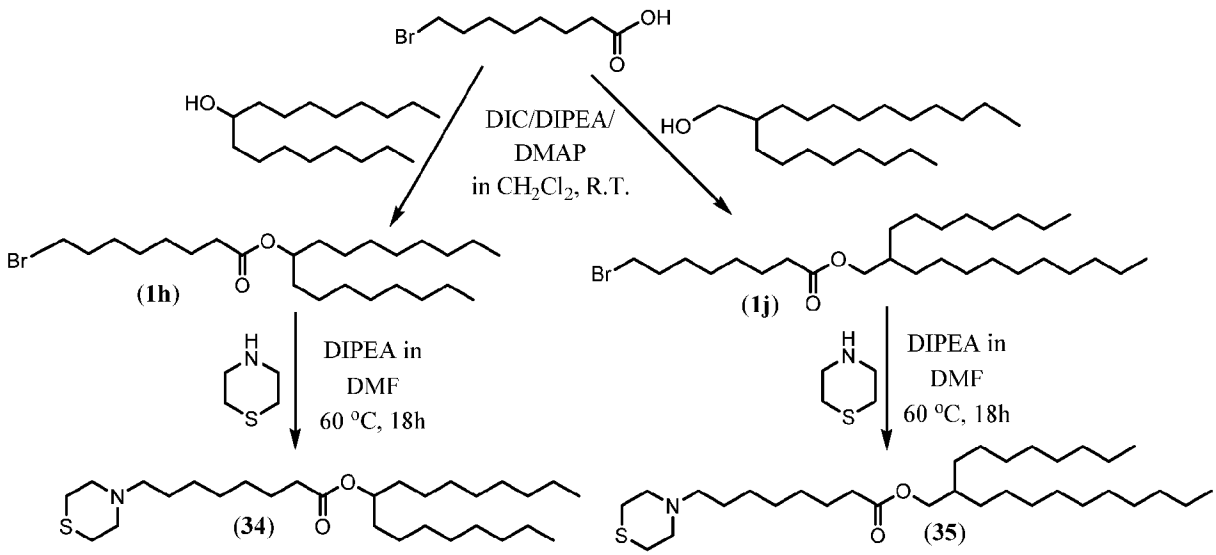
A mixture of diol (**1g**) (0.266g, 1.21 mmol.), oleoyl chloride (1.09g, 1.20mL, 3 eq.) and di-isopropyl ethyl amine (1.24mL, 6eq.) in dry methylene chloride (15 mL) was stirred under nitrogen atmosphere at room temperature for 24h. Reaction progress was monitored using TLC. The solvent was then evaporated under high vacuum and the crude product (**39**) purified with flash chromatography on silica.

[0234] Synthesis of 3-thiomorpholinopropane-1,2-diyl (9Z, 9'Z, 12Z, 12'Z)-bis-linoleate (40)

A mixture of diol (**1g**) (0.266g, 1.21 mmol.), linoleoyl chloride (1.09g, 1.17mL, 3 eq.) and di-isopropyl ethyl amine (1.24mL, 6eq.) in dry methylene chloride (15 mL) was stirred under nitrogen atmosphere at room temperature for 24h. Reaction progress was monitored using TLC. The solvent was then evaporated under high vacuum and the crude product (**40**) purified with flash chromatography on silica.

[0235] General Procedure for the preparation of the hydrochloride salt derivatives.

The free-base lipid compounds were dissolved in anhydrous methylene chloride and a 1.1 equivalent solution of 4N hydrochloric acid in 1,4-dioxane was added. The mixture was stirred at room temperature between 30min to 1h. The solvent was then evaporated, and the crude product purified using reverse phase preparative HPLC using water-methanol gradient. Pure hydrochloride salt derivative compounds **39a**, **40a**, and **41a** were characterized by UPLC and LC/MS.



Scheme 7

[0236] Synthesis of heptadecane-9-yl 8-bromooctanoate (1h)

To a solution of 8-bromooctanoic acid (1.04g, 4.6mmol) and heptadecane-9-ol (1.5g, 5.8 mmol) in dichloromethane (20mL) was added *N,N'*-Diisopropylcarbodiimide (DIC) (0.9 mL, 0.732 g, 5.8 mmol), diisopropyl ethyl amine (DIPEA) (3.3 mL, 18.7 mmol) and 4-(Dimethyl amino) pyridine (DMAP) (114 mg, 0.9 mmol). The reaction was allowed to stir at room temperature for 18h under nitrogen atmosphere, after which the reaction mixture was diluted

with 200 mL dichloromethane and washed 3 times with saturated sodium bicarbonate (3 x 50 mL). The organic layer was then and washed with brine (3 x 50 mL) and dried over sodium sulfate. The organic layer was filtered off and solvent evaporated. The crude product (**1h**) was purified by flash chromatography on silica using acetonitrile-hexane 2:8.

LC/MS analysis: $(M + Na)^+$ m/z 483.2.

[0237] Synthesis of 2-octyldodecyl 8-bromooctanoate (1j)

An analogous procedure was followed as for compound (**1h**)

LC/MS analysis: $(M + Na)^+$ m/z 525.3

[0238] Synthesis of heptadecane-9-yl 8-thiomorpholinooctanoate (34)

Heptadecane-9-yl 8-bromooctanoate (**1h**) (0.7g, 1.4 mmol) was dissolved in 20mL of dry DMF. Thiomorpholine (0.417 mL) and diisopropyl ethyl amine (0.483 mL) were added. The flask was purged of air with nitrogen and the reaction mixture stirred at 62 °C for 18h. The solvent was evaporated, and the residue was taken up in dichloromethane (10 mL). Insoluble material was filtered out and the organic layer was diluted then washed with saturated bicarbonate solution followed by a washing with brine. The aqueous layers were back-extracted once with dichloromethane. The organic layers were then combined and dried over anhydrous sodium sulfate. The drying agent was filtered out and solvent was evaporated under reduced pressure. The crude product was purified by chromatography on silica eluting with a mixture of acetonitrile-hexane 20-80.

LC/MS analysis: MH+, m/z 484.4

[0239] Synthesis of heptadecane-9-yl 8-thiomorpholinooctanoate (35)

An analogous procedure was followed as for compound (**34**).

LC/MS analysis: MH+, m/z 526.4

[0240] General Procedure for the preparation of the hydrochloride salt derivative.

The free-base lipid compounds **34** and **35** were dissolved in anhydrous methylene chloride and a 1.1 equivalent solution of 4N hydrochloric acid in 1,4-dioxane was added. The mixture was stirred at room temperature between 30min to 1h. The solvent was then evaporated, and the crude product purified using reverse phase preparative HPLC using water-methanol gradient. Pure hydrochloride salt derivative compounds **34a** and **35a** were characterized by UPLC and LC/MS.

EXAMPLE 2: Thiomorpholine Lipid Nanoparticles

[0241] Based on a systematic Design of Experiment (DOE) approach, compositions including Thiomorpholines and helper lipids were made and complexed with mRNA. As shown in Table 1, the formulations examined varied in molar ratios of thiomorpholines and helper lipids.

Table 1. Exemplary lipid nano particle formulations

LNP	Lipid	Cationic lipid	DOPE	Cholesterol	DMG-PEG	Peptide SEQ ID NO: 47	N/P Ratio
1	Compound **34a** (salt)	40.00%	16.00%	42.40%	1.60%	no	10
2	Compound **34a** (salt)	20.00%	34.00%	44.40%	1.60%	no	10
3	Compound **34a** (salt)	40.00%	16.00%	42.40%	1.60%	no	30
4	Compound **34a** (salt)	60.00%	12.00%	26.40%	1.60%	no	50
5	Compound **34a** (salt)	60.00%	12.00%	26.40%	1.60%	20ug/ml	50
6	Compound **35a** (salt)	40.00%	16.00%	42.40%	1.60%	no	10
7	Compound **35a** (salt)	20.00%	34.00%	44.40%	1.60%	no	10
8	Compound **35a** (salt)	40.00%	16.00%	42.40%	1.60%	no	30
9	Compound **35a** (salt)	60.00%	12.00%	26.40%	1.60%	no	50
10	Compound **35a** (salt)	60.00%	12.00%	26.40%	1.60%	20ug/ml	50
11	Compound **34** (free base)	20.00%	34.00%	44.40%	1.60%	no	10
12	Compound **34** (free base)	60.00%	12.00%	26.40%	1.60%	no	50
13	Compound **35** (free base)	20.00%	34.00%	44.40%	1.60%	no	10
14	Compound **35** (free base)	60.00%	12.00%	26.40%	1.60%	no	50

[0242] Lipid nano particle (LNP) formulations were screened and assessed by *in vivo* functional testing using the RNA payload of the complex. Performance and transfection efficiency analyses included payload delivery, biodistribution, and expression of the payload-encoded protein.

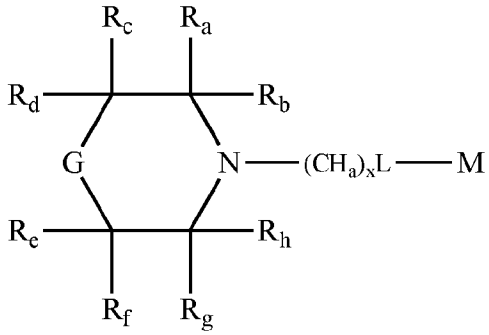
[0243] All the LNP formulations contained one of the thiomorpholines and DOPE, Cholesterol, and DMG-PEG. Some formulations had peptide SEQ ID NO: 47. All the lipids were weighed and solubilized in ethanol at the desired molar ratio. This lipid mix and firefly luciferase (fLuc) mRNA were complexed into LNPs using a microfluidic device. The LNPs were dialyzed in phosphate buffer and particle size and homogeneity were measured using dynamic light scattering. The LNPs were injected in mice the next day.

[0244] Female BALB/c mice aged 6-10 weeks old were purchased from The Jackson Laboratory and were acclimatized for 7 days before the study. Mice were injected with LNPs equivalent to 10 µg fLuc mRNA using intravenous tail vein injection in a total volume of 200µl. At 4 h post-injection, mice were anesthetized with isofluorane anesthesia and imaged 10 min after intraperitoneal injection of 100 µL Rediject D-Luciferin (Perkin Elmer). Bioluminescence imaging was quantified in vivo (whole body) and ex vivo (organ) using an IVIS Lumina III imaging system (Perkin Elmer) and analyzed using Living Image software.

[0245] All the LNP formulations with thiomorpholines had particle size >120nm, polydispersity index < 0.3, (Fig. 1) and % encapsulation efficiency (%EE) > 70% except formulation ID 12 (Fig. 2). Intravenous administration of the LNP formulations with thiomorpholines resulted in mRNA delivery and luciferase expression distributed between the liver (Fig. 3) and the spleen (Fig. 4) of the injected mice.

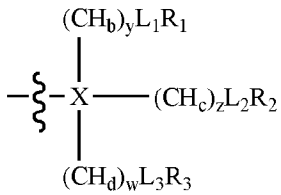
WHAT IS CLAIMED IS:

1. A compound having a structure selected from (I):.



I

where M is



G is O or a thiol-group selected from the group consisting of -S-, -SO-, -$S(O)_2$-, and -$S(CH_2)$-

L is selected from the group consisting of O, -C(=O)-, -$C(=O)N(R_4)$-, -$N(R_4)C(=O)$-, -$N(R_4)C(=O)N(R_5)$-, C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -$(R_6)P(=O)$- and a bond;

X is selected from the group consisting of C and N;

each R_a, R_b, R_c, R_d, R_e, R_f, R_g and R_h are independently selected from the group consisting of H, unbranched C_1-C_{12} alkyl groups, branched C_1-C_{12} alkyl groups, halogenated unbranched C_1-C_{12} alkyl groups, halogenated branched C_1-C_{12} alkyl groups, a C_3-$_7$ membered alkyl ring, a C_3-C_7 carbocyclic ring formed from two R groups attached

to an individual atom and a C_3-C_7 carbocyclic ring formed from R groups on two adjacence ring carbon atoms;

L_1, L_2 and L_3 are independently selected from the group consisting of O, -C(=O)-, -C(=O)N(R_6)-, -N(R_6)C(=O)-, -N(R_6)C(=O)N(R_7)-, C(=O)O- , -OC(=O)-,-S-, -S-S-,-C=S-, -C(=S)O-, -C(=O)S-, -SC(=O)-, -(R_6)P(=O)- and a bond;

R_1, R_2 and R_3 are independently selected from the group consisting of H, unbranched C_1-C_{25} alkyl groups, branched C_1-C_{25} alkyl groups, halogenated unbranched C_1-C_{25} alkyl groups, and halogenated branched C_1-C_{25} alkyl groups;

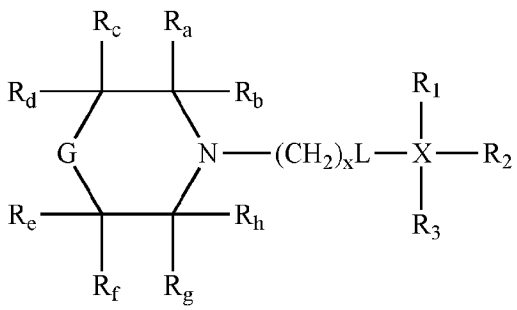
each occurrence of R_4, R_5, R_6, and R_7 are independently selected from the group consisting of H and a C_1-C_6 alkyl;

w, x, y and z are selected from the group of integers selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12;

-(CH_a)$_x$-, -(CH_b)$_y$-, -(CH_c)$_z$- and -(CH_d)$_w$- are selected from C_1-C_{12} alkyl, C_2-C_{12} alkene, and the absence of the methylene when w, x, y or z is 0; and

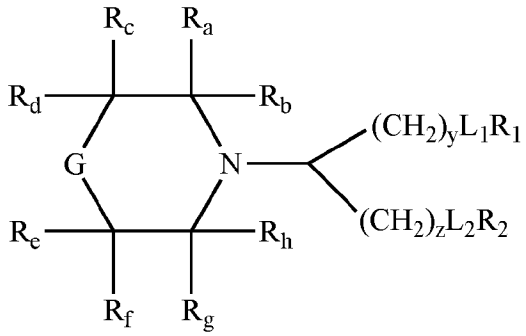
a, b, c, and d is 2 for C_1-C_{12} alkyl groups and selected from 1 and 2 for C_1-C_{12} alkene groups.

2. The compound of claim 1, having the structure of compound I-A:



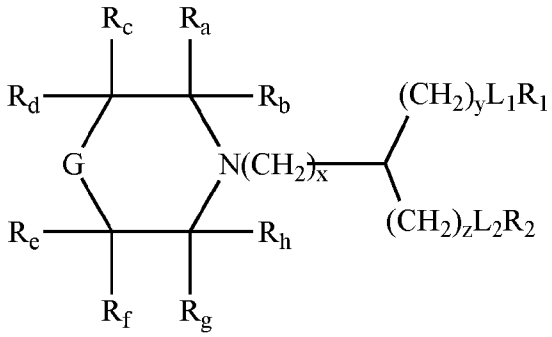
I-A.

3. The compound of claim 1, having the structure of I-B:



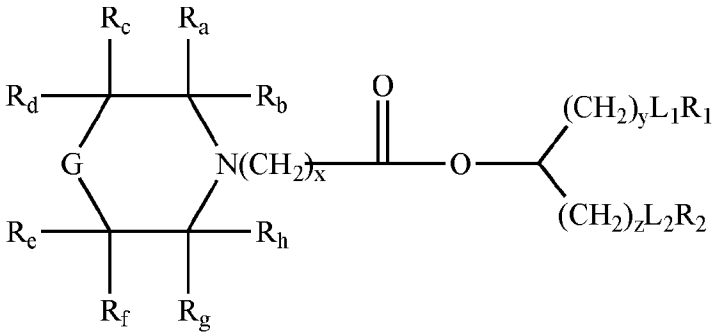
I-B.

4. The compound of claim 1, having the structure of I-C:



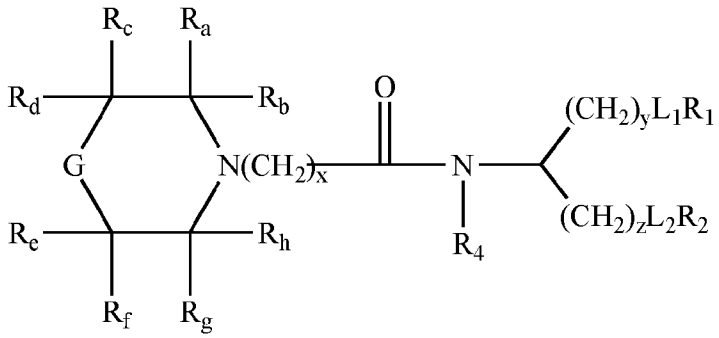
I-C.

5. The compound of claim 1, having the structure of I-D:



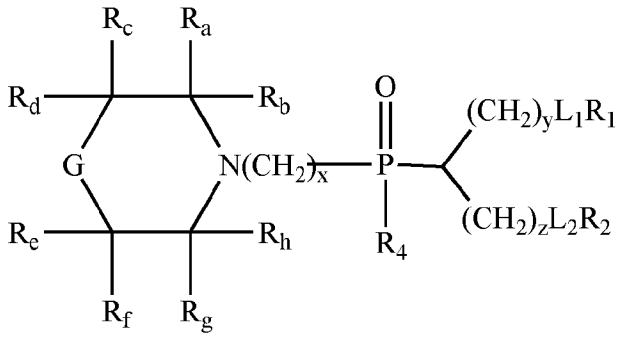
I-D.

6. The compound of claim 1, having the structure of I-E:



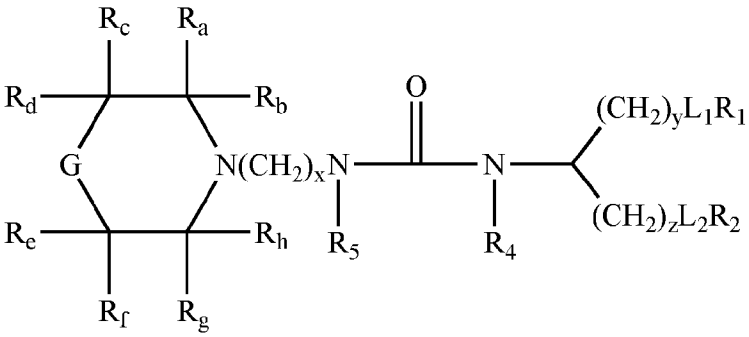
I-E.

7. The compound of claim 1, having the structure of I-F:



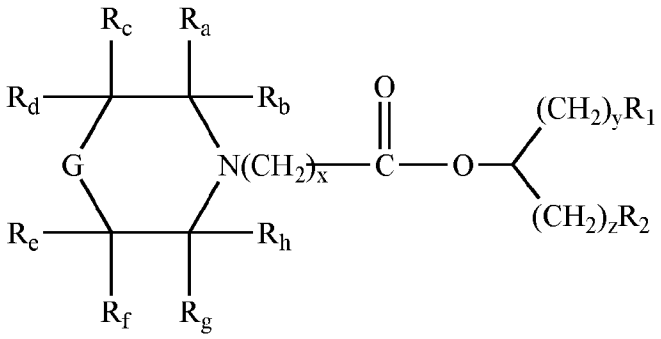
I-F.

8. The compound of claim 1, having the structure of I-G:



I-G.

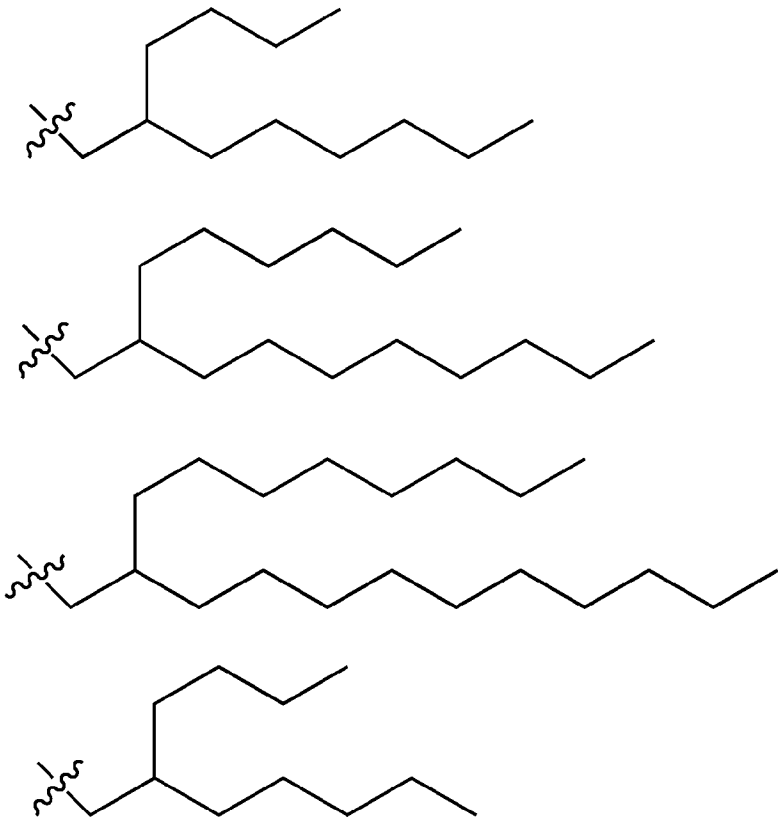
9. The compound of claim 1, having the structure of I-H:

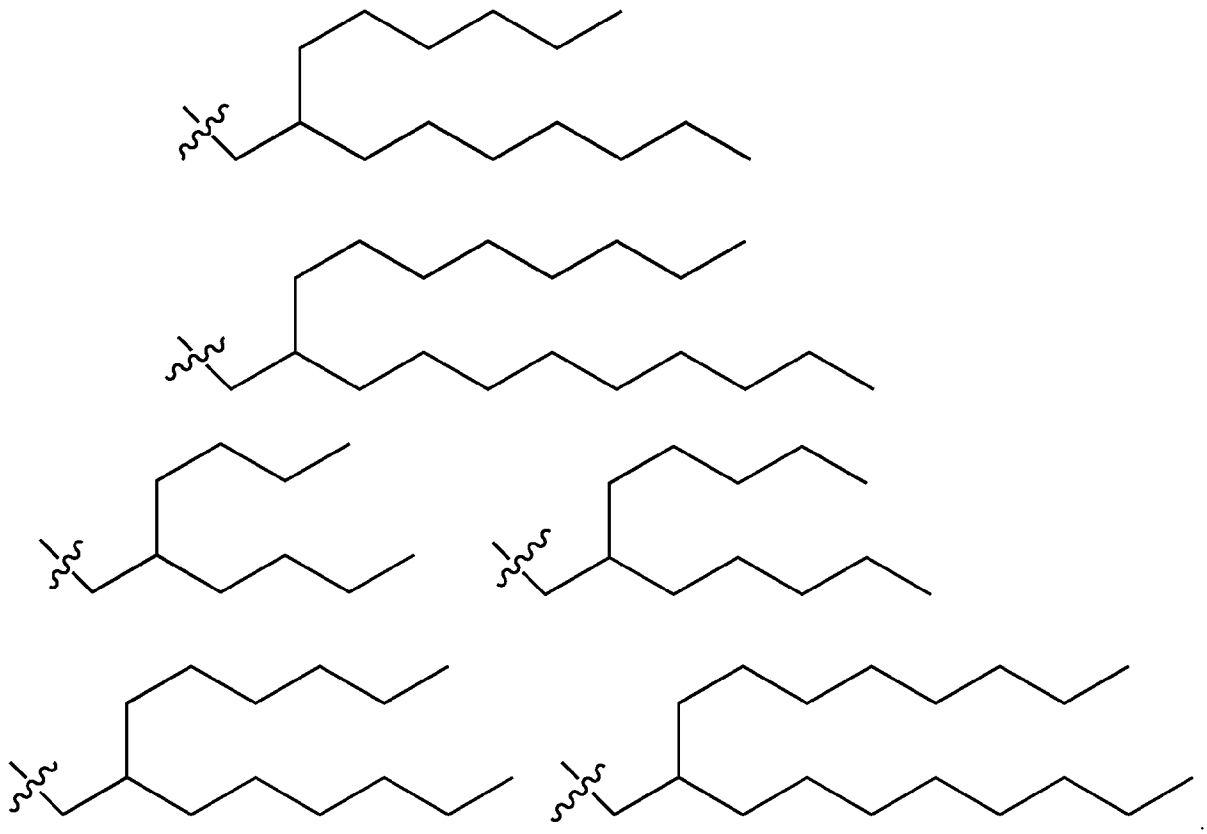


I-H.

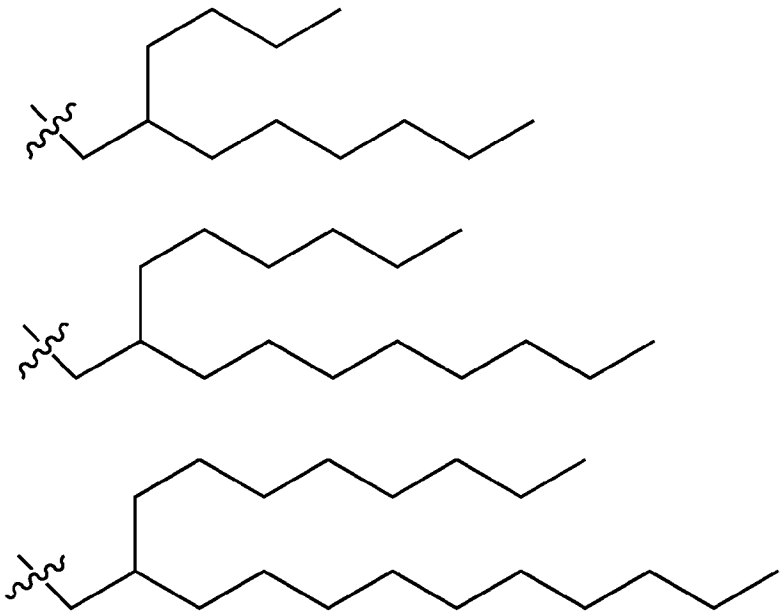
10. The compound of claim 1, wherein each of R_1, R_2 and R_3 are independently selected from the group consisting of H, a diene and a triene.

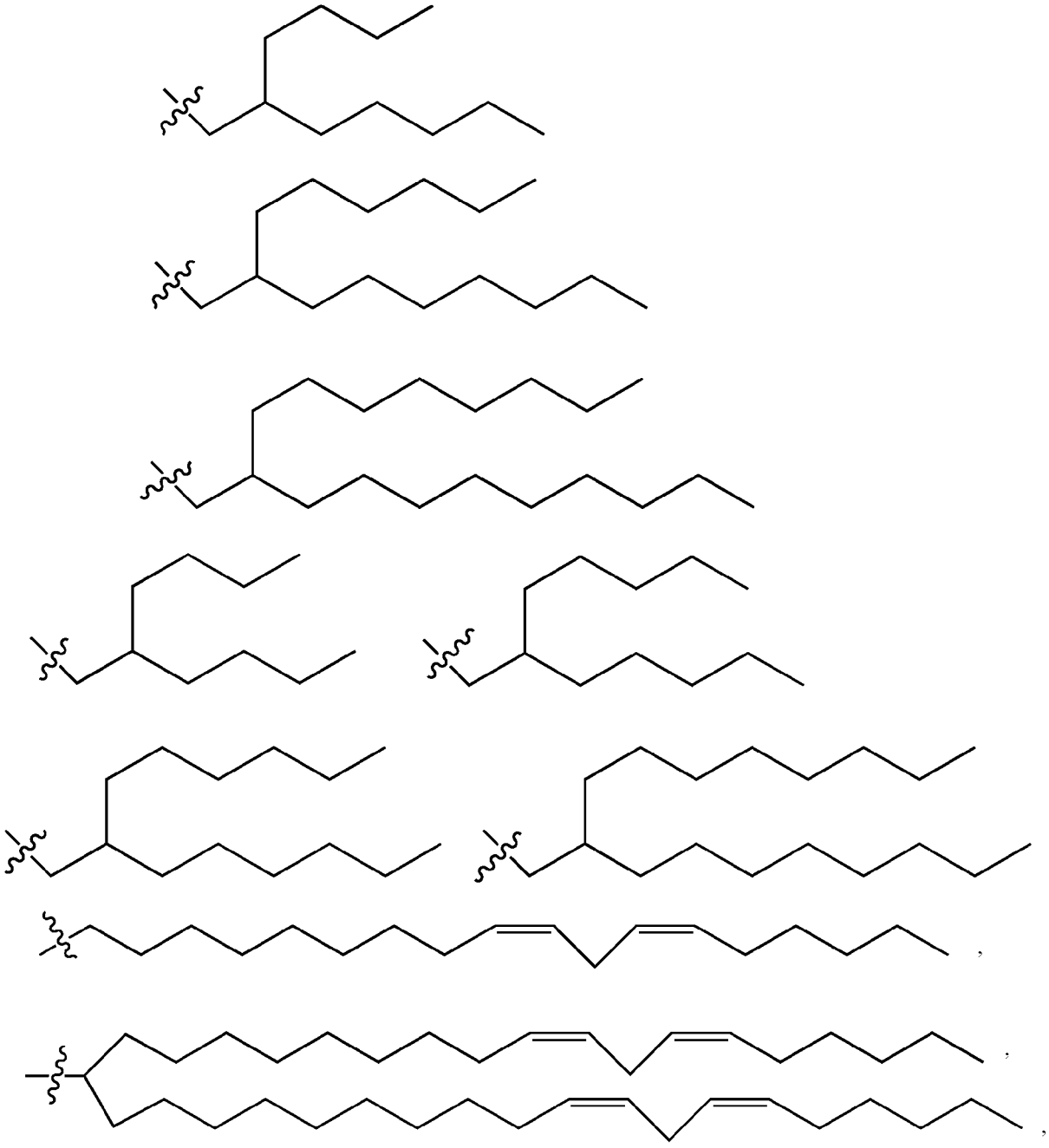
11. The compound of claim 1, wherein M has a structure selected from the group consisting of

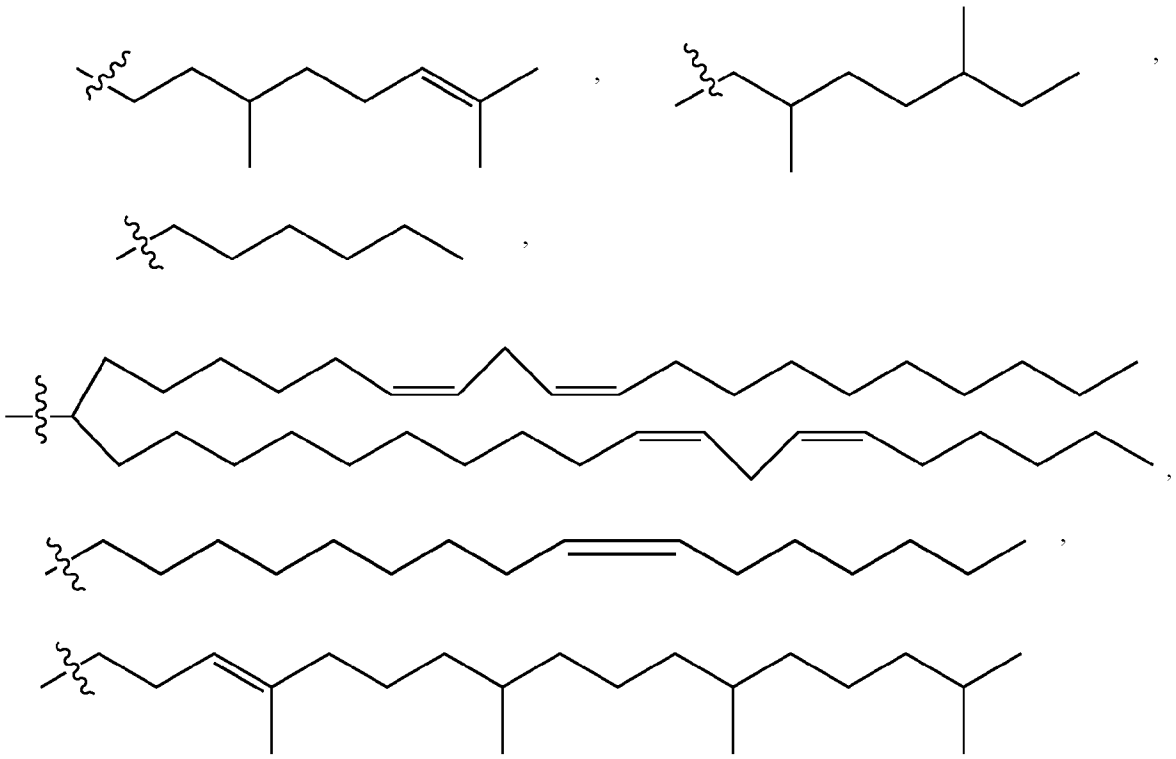




12. The compound of claim 1, wherein each R_1, R_2 and R_3 is independently selected from the group consisting of H,







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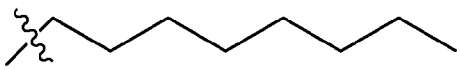
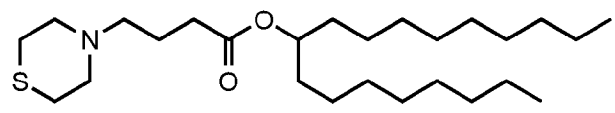


13. The compound of claim 1 or 11, wherein the compound is selected from the group consisting of:



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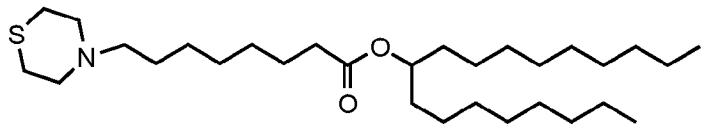

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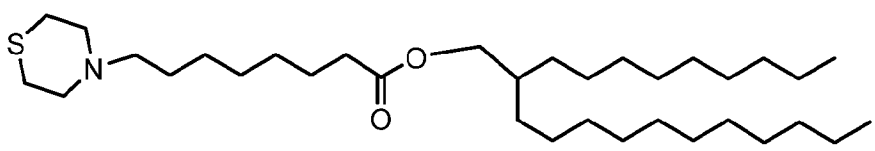

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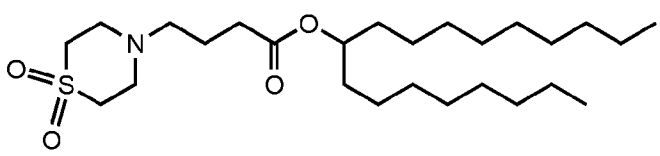

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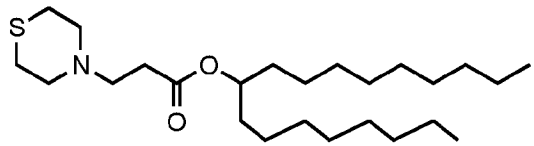

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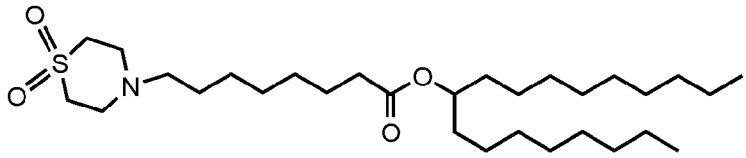

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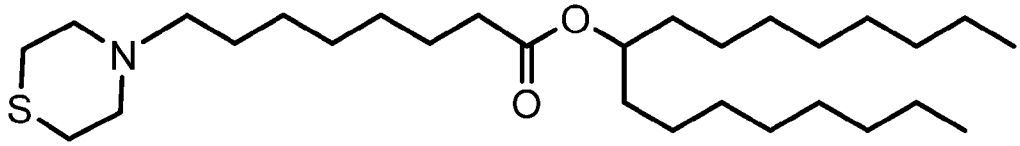

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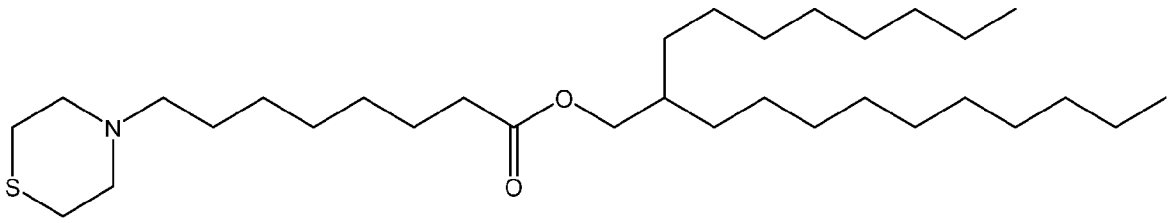

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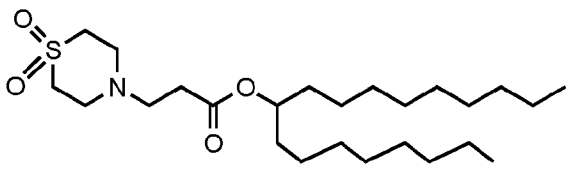
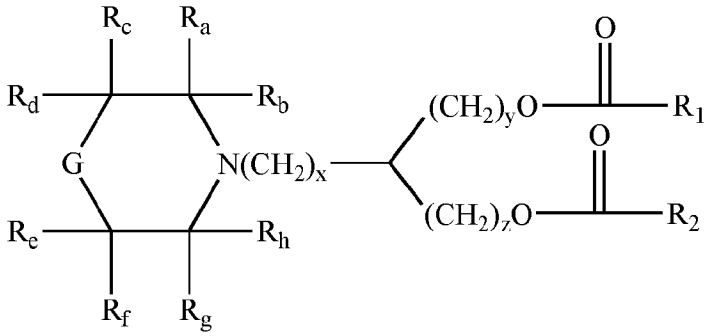


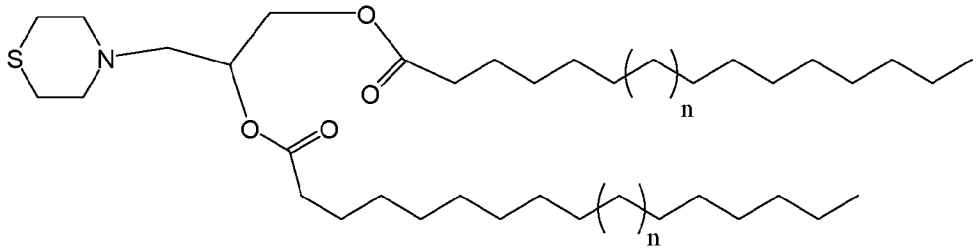
7.

14. The compound of claim 1, having the structure of I-J:



I-J.

15. The compound of claim 14, wherein the compound is selected from the group consisting of



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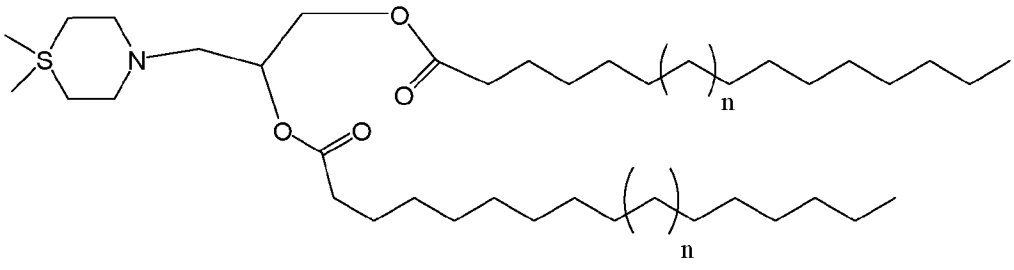

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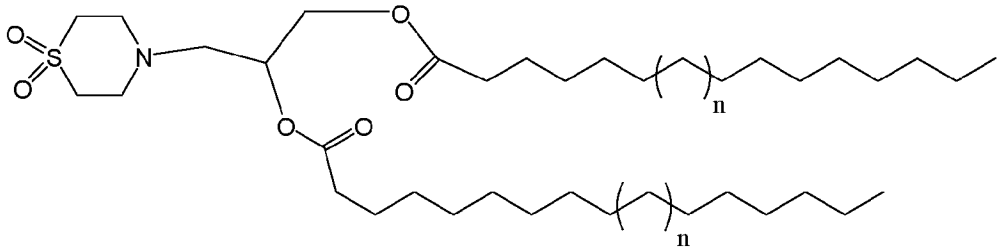

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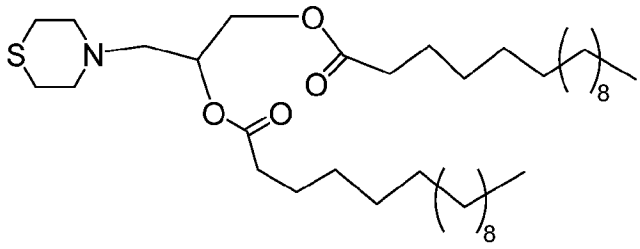
16. The compound of claim 14, wherein the compound is selected from the group consisting of:



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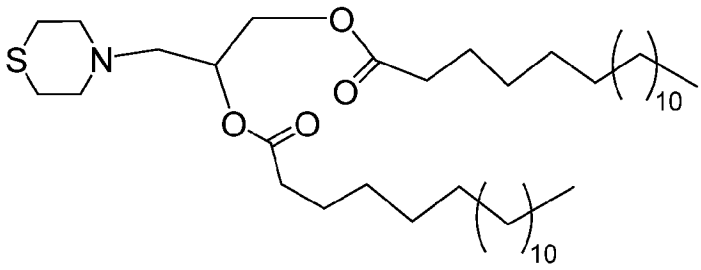

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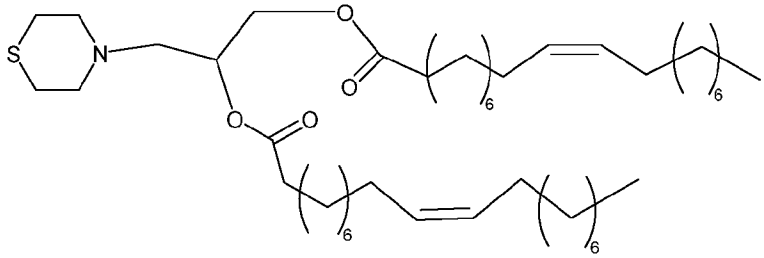

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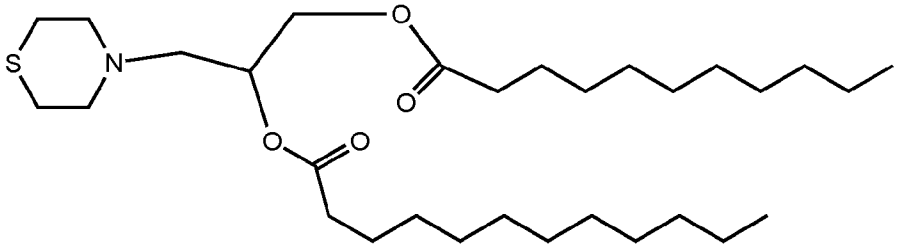

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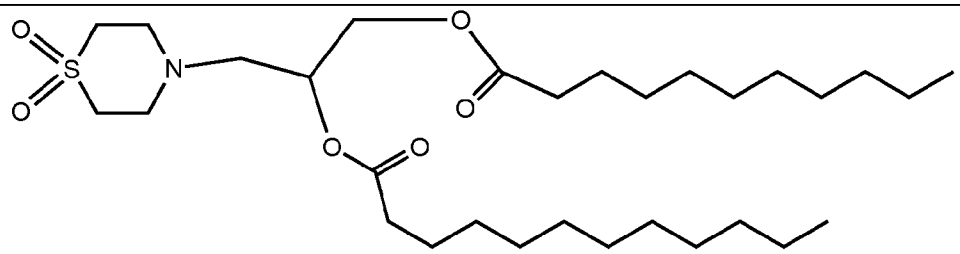

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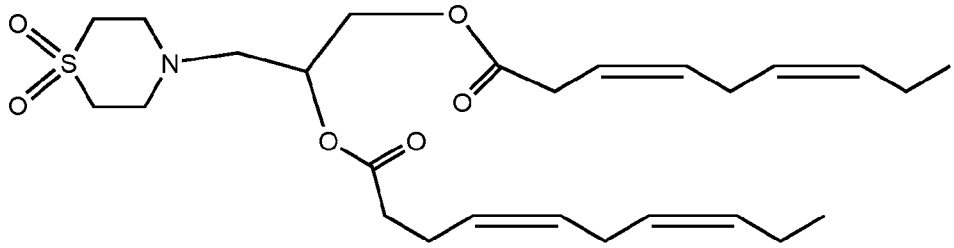

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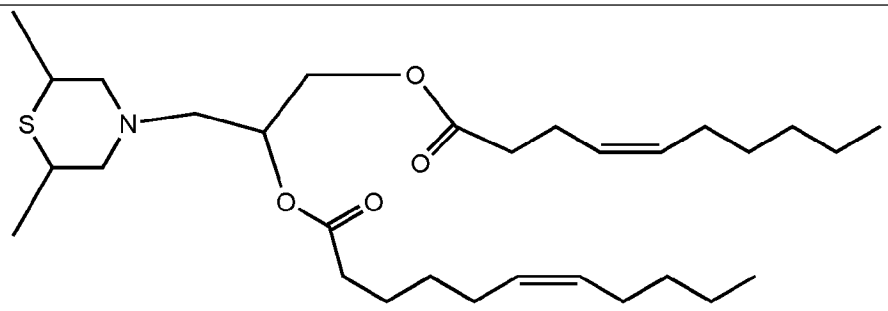

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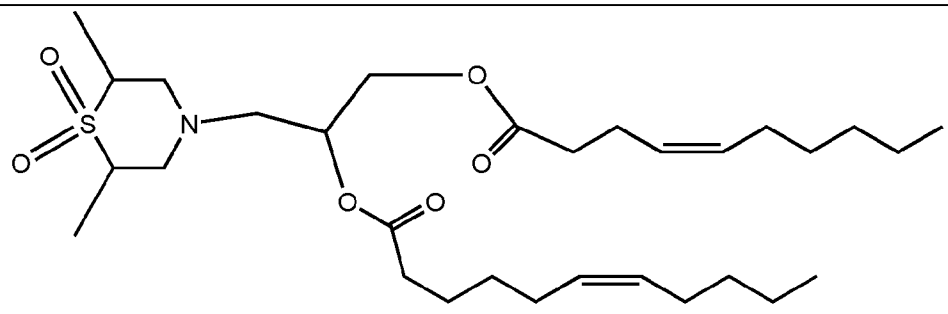

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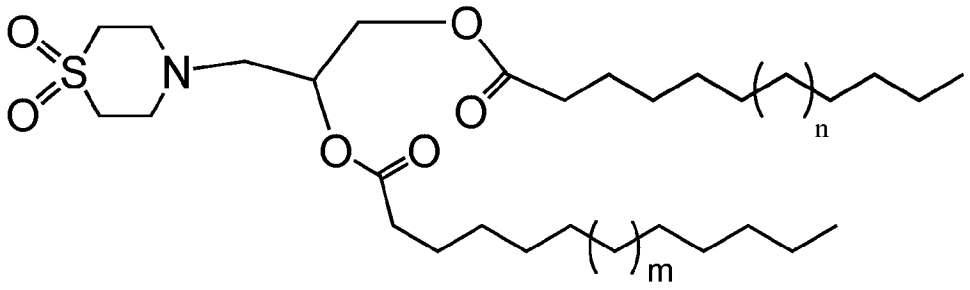

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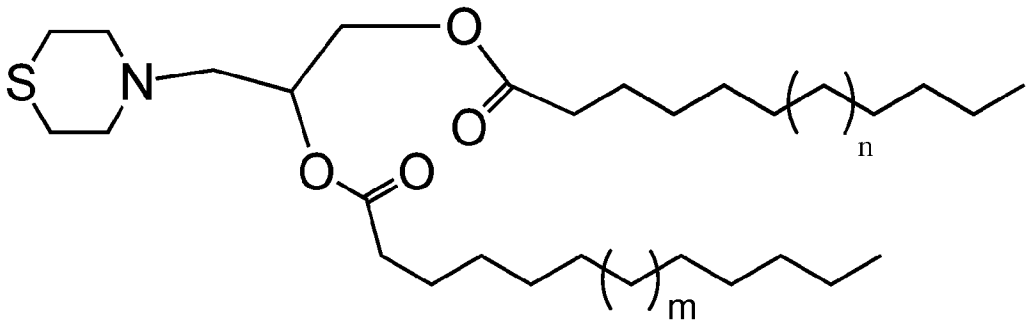
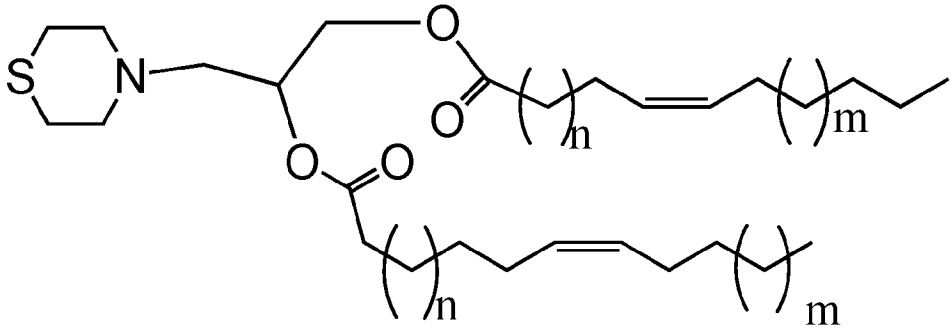


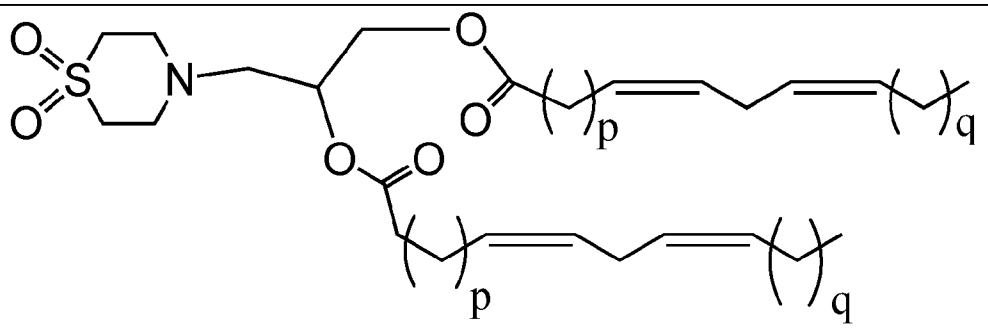
n = m = 5

22



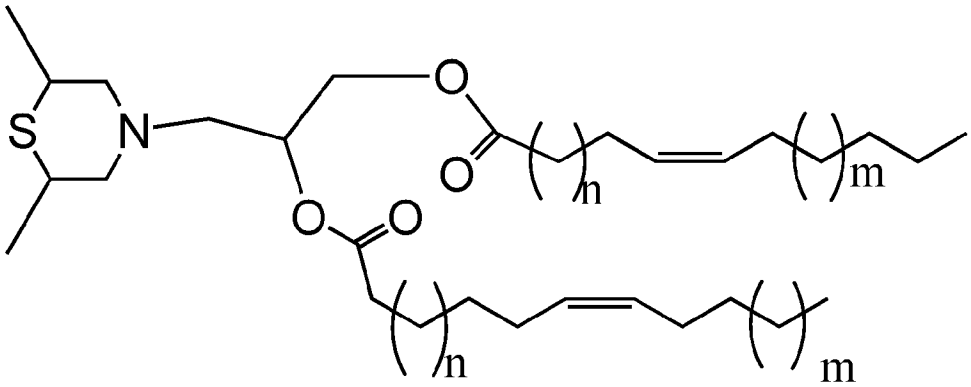
n = m = 5

23



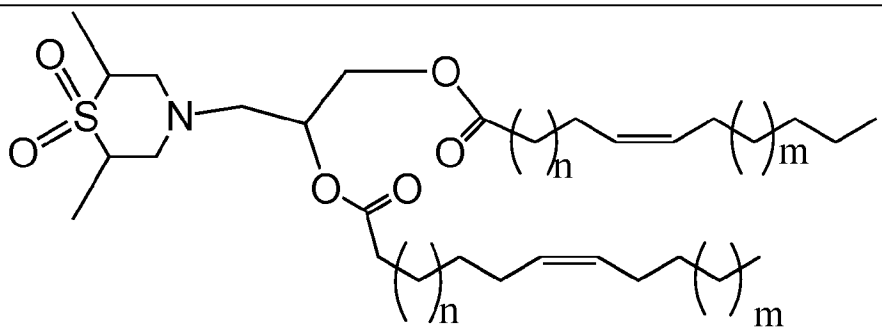
p = q = 5

24



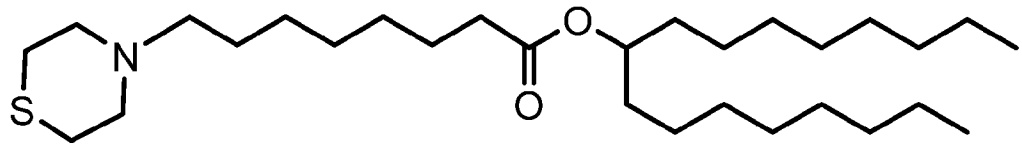
n = m = 5

25

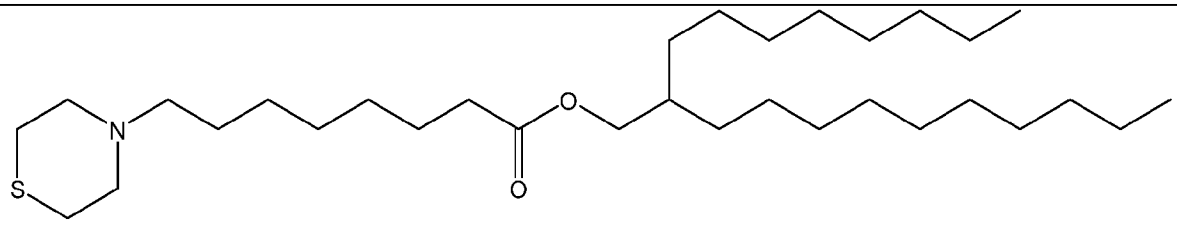


n = m = 5

26



34



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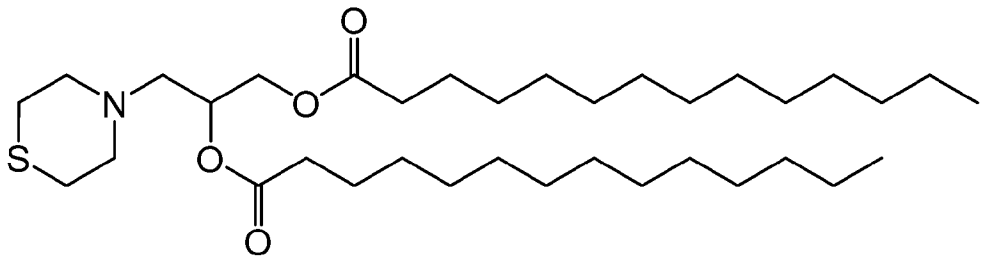

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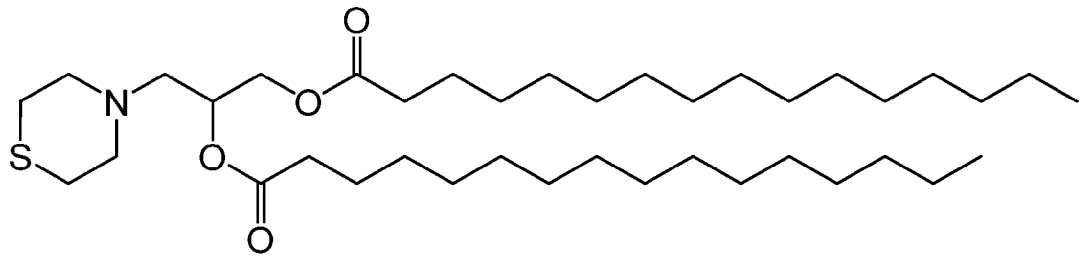
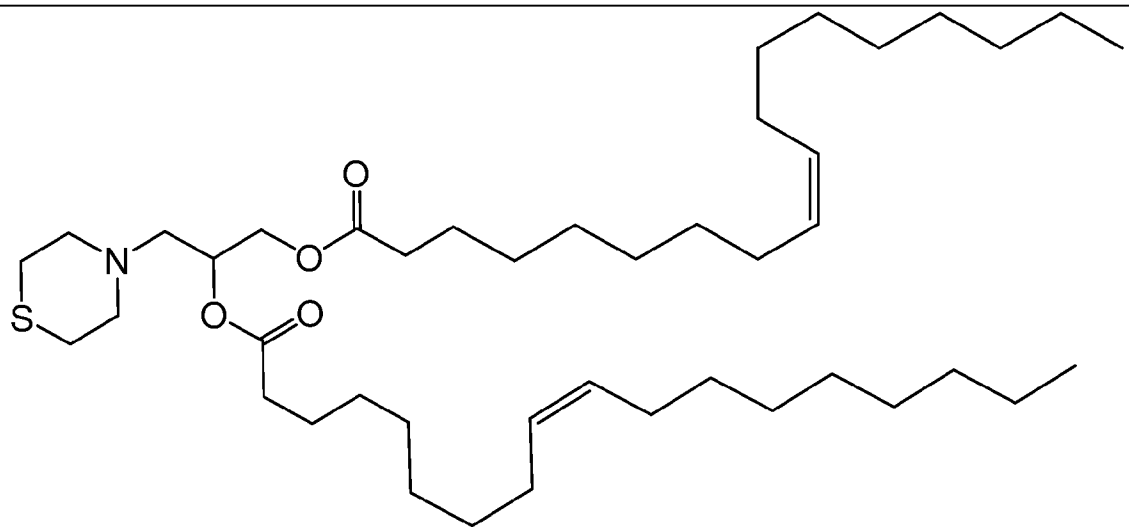


37



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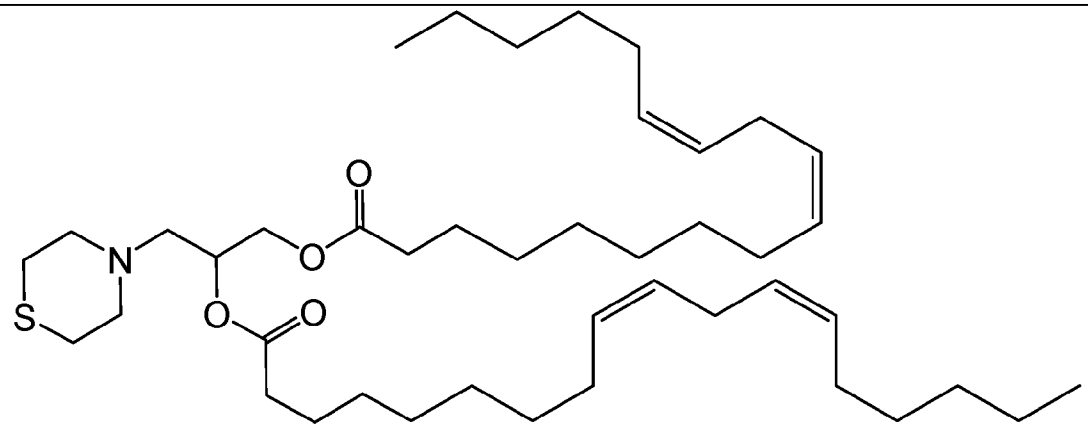

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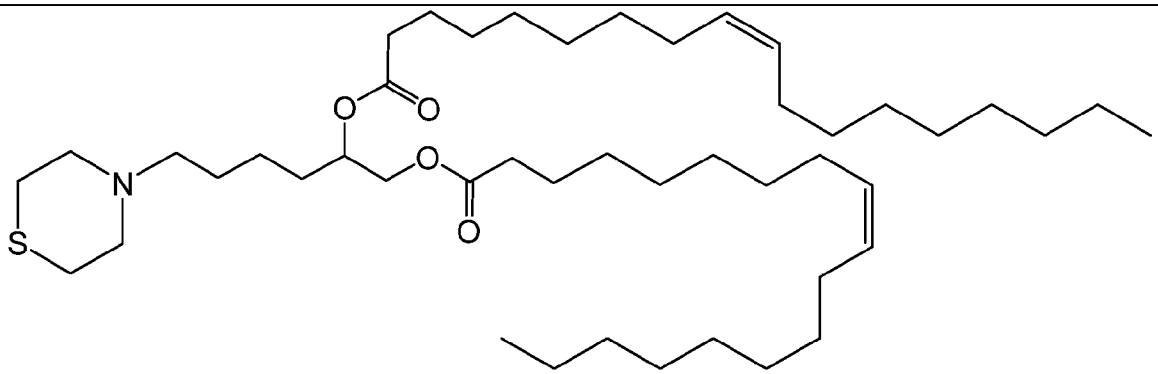

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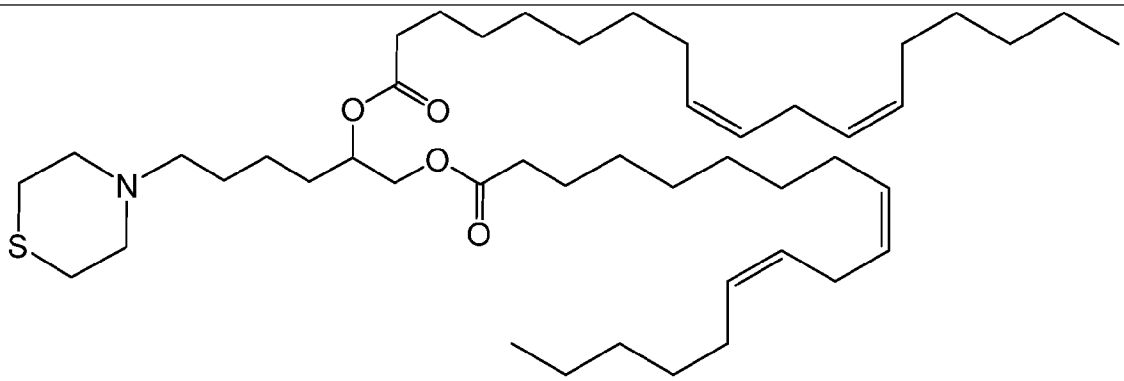

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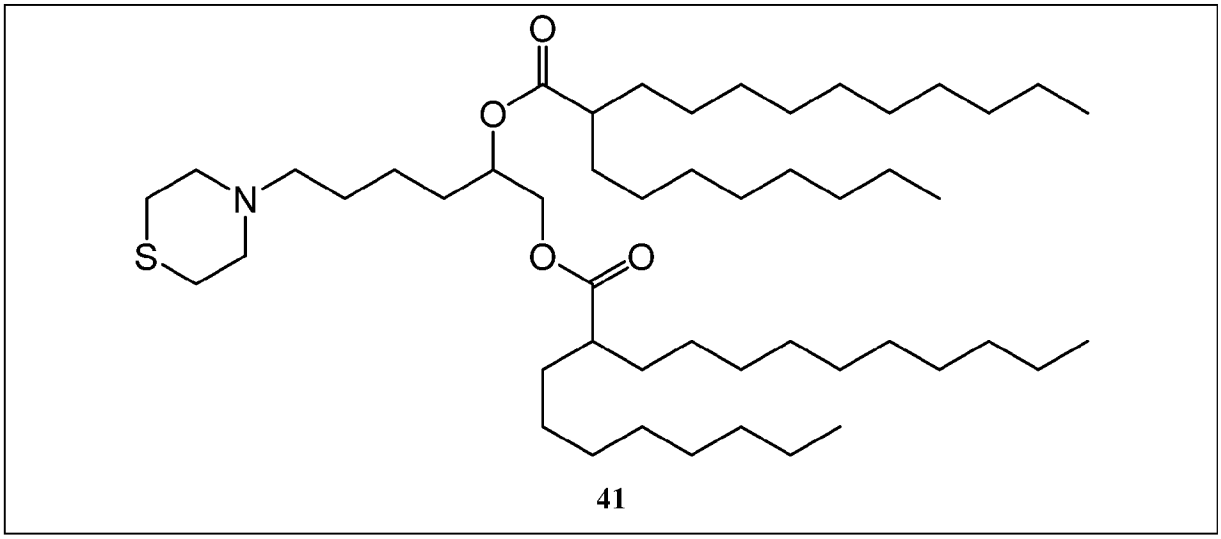


41

17. The compound of claims 1 or 8, wherein one of L_1R_1 and L_2R_2 is an unsaturated fatty acid.

18. The compound of claims 1 or 8, wherein both L_1R_1, and L_2R_2, are an unsaturated fatty acid moiety.

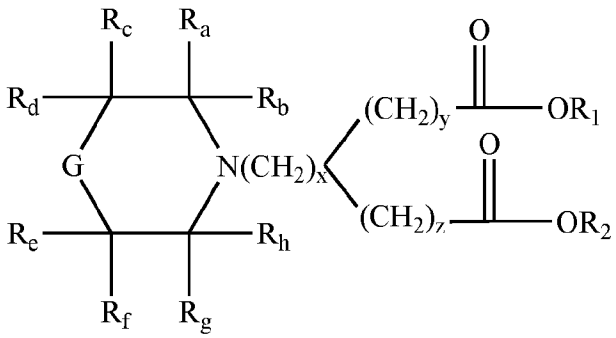
19. The compound of claim 17 or 18, wherein the unsaturated fatty acid moiety is selected from the group consisting of: α-linolenic acid (C 18:3), stearidonic acid (C 18:4), eicosapentaenoic acid (C 20:5), cervonic acid (C 22:6), linoleic acid (C 18:2), linolelaidic acid (C 18:2), γ-linolenic acid (C 18:3), dihomo-γ-linolenic acid (C 20:3), arachidonic acid (C 20:4), docosatetraenoic acid (C 22:4), palmitoleic acid (C 16:1), vaccenic acid (C 18:1), paullinic acid (C 20:1), oleic acid (C 18:1), elaidic acid (C 18:1), gondoic acid (C 20:1), erucic acid (C 22:1), nervonic acid (C 24:1), and mead acid (C 20:3).

20. The compound of claims 1 or 8, wherein one of L_1R_1 and L_2R_2 is an saturated fatty acid.

21. The compound of claims 1 or 8, wherein both L_1R_1, and L_2R_2, are an saturated fatty acid moiety.

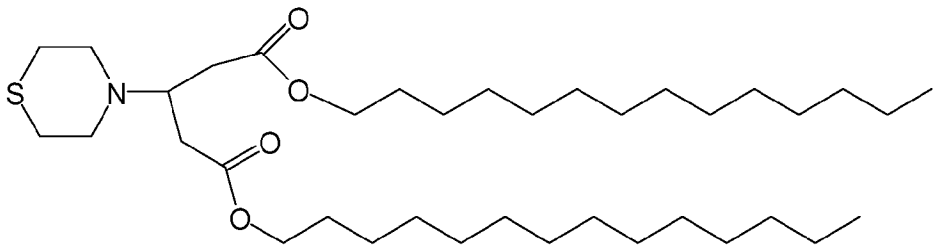
22. The compound of claim 20 or 21, wherein the saturated fatty acid moiety is selected from the group consisting of propionic acid (C 3:0), butyric acid (C 4:0), valeric acid (C 5:0), caproic acid (C 6:0), enanthic acid (C 7:0), caprylic acid (C 8:0), pelargonic acid (C 9:0), capric acid (C 10:0), undecylic acid (C 11:0), lauric acid (C 12:0), tridecylic acid (C 13:0), myristic acid (C 14:0), pentadecylic acid (C 15:0), palmitic acid (C 16:0), margaric acid (C 17:0), stearic acid (C 18:0), nonadecylic acid (C 19:0), arachidic acid (C 20:0), heneicosylic acid (C 21:0), behenic acid (C 22:0), tyricosylic acid (C 23:0), lignoceric acid (C 24:0) and pentacosylic acid (C 25:0).

23. The compound of claim 1, having the structure of I-K:

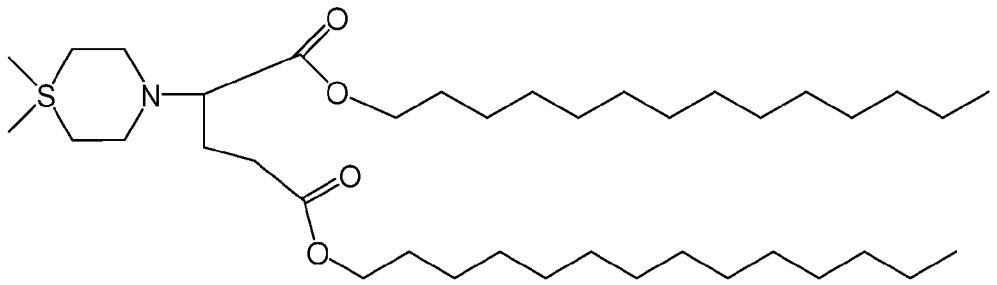


I-K.

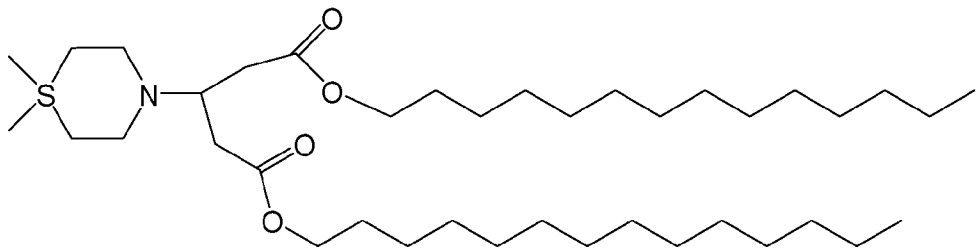
24. The compound of claim 23, wherein the compound is selected from the group consisting of:



12,

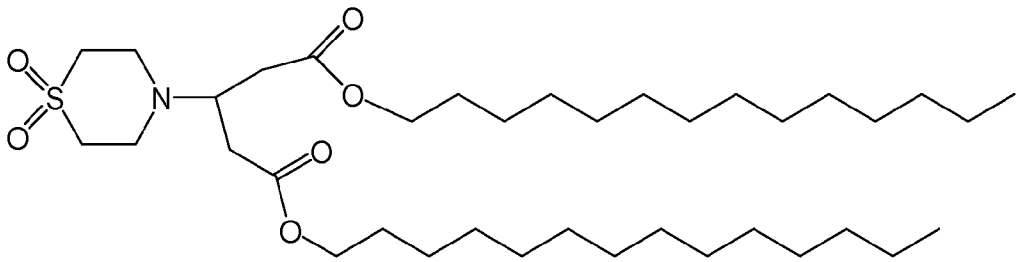


13,



14,

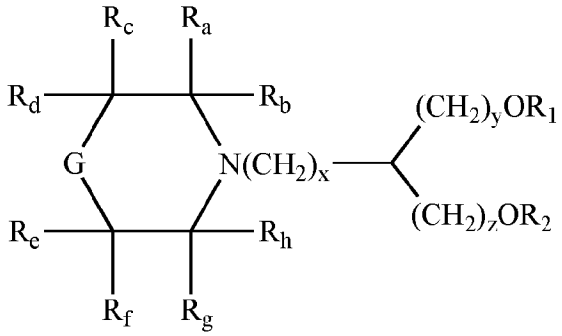
and



15.

25. The compound of claim 1 or 23, wherein the ester moieties -OR_1 and –OR_2 are derived from an alcohol selected from the group consisting of: tert-butyl alcohol (C4), tert-amyl alcohol (C 5), 3-methyl-3-pentanol (C 6), 1-hexanol (C 6), 1-heptanol (C 7), 1-octanol (C 8), 1-nonanol (C 9), 1-decanol (C 10), 1-undecanol (C 11), 1-dodecanol (C 12), 1-tridecanol (C 13), 1-tetradecanol (C 14), 1-pentadecanol (C 15), 1-hexadecanol (C 16), cis-9-hexadecen-1-ol (C 17), 1-n-heptadecanol (C 18), 1-octadecanol (C 19), 1-octadecenol (C 20), 1-nonadecanol (C 21) and 1-eicosanol (C 22).

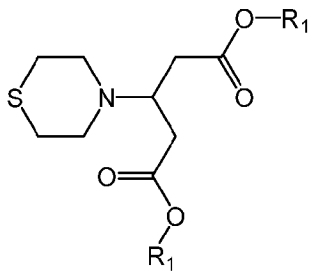
26. The compound of claim 1, having the structure of I-L:



I-L.

27. The compound of claim 1 or 23, wherein R_1 and R_2 are selected from the group consisting of C_1-C_{22} alkyl and C_2-C_{22} alkene groups.

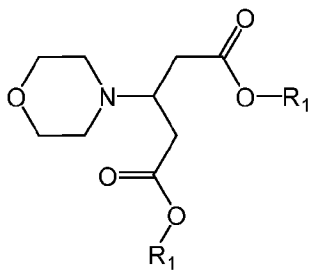
28. The compound of claim 1, having the structure of formula (II)



(II)

wherein R_1 is selected from Myristyl ($C_{14}H_{29}$), Palmitoyl ($C_{16}H_{33}$), Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9), Oleyl ($C_{18}H_{37}$, cis-Δ^9), and Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12}).

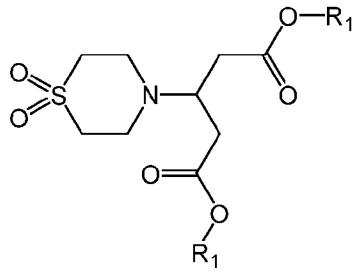
29. The compound of claim 1, having the structure of formula (III)



(III)

wherein R_1 is selected from Myristyl ($C_{14}H_{29}$), Palmitoyl ($C_{16}H_{33}$), Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9), Oleyl ($C_{18}H_{37}$, cis-Δ^9), and Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12}).

30. The compound of claim 1, having the structure of formula (IV)



(IV)

wherein R_1 is selected from Myristyl ($C_{14}H_{29}$), Palmitoyl ($C_{16}H_{33}$), Palmitoleyl ($C_{16}H_{31}$, cis-Δ^9), Oleyl ($C_{18}H_{37}$, cis-Δ^9), and Linoleyl ($C_{18}H_{35}$, cis, cis-Δ^9, Δ^{12}).

31. A composition comprising the compound of any one of claims 1 to 30 and a payload.

32. The composition of claim 31, further comprising one or more ionizable lipids.

33. The composition of claim 31 or 32, further comprising a helper lipid.

34. The composition of any one of claims 31 to 33, further comprising a stabilizer.

35. The composition of claim 34, wherein the stabilizer is selected from the group consisting of a PEGylated lipid and a surfactant.

36. The composition of claim 31, wherein the payload is a nucleic acid, a protein, or a ribonucleoprotein.

37. The composition of clam 31, wherein the payload is a nucleic acid.

38. The composition of clam 37, wherein the nucleic acid is a ribonucleic acid.

39. The composition of claim 38, wherein the ribonucleic acid is selected from antisense RNA, mRNA, o-RNA, miRNA, siRNA, or any combination thereof.

40. The composition of any one of claims 31 to 39, further comprising a delivery-enhancing peptide.

41. The composition of claim 40, wherein the delivery-enhancing peptide is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 583.

42. The composition of any one of claims 31 to 41, further comprising an exosome, or one or more biological materials derived or purified from exosomes.

43. A method of delivering a payload to a cell, comprising contacting the cell with the composition of any one of claims 31 to 42.

44. The method of claim 43, wherein the cell is a mammalian cell.

45. The method of claim 43, wherein the contacting is performed *in vitro*, *ex-vivo*, or *in vivo*.

46. A method for administering a therapeutic agent to a patient in need thereof, the method comprising:

preparing or providing the composition of any one of claims 31 to 42; and

administering the composition to the patient.

47. Use of a composition according to any of claims 31 to 42 in the manufacture of a medicament.

48. A composition comprising:

(i) one or more compounds according to claims 1 to 30, and

(ii) one or more of a structural lipid, an ionizable lipid, and a stabilizing agent; and

(iii) optionally, a payload.

49. The composition according to claim 48, comprising:

(i) a compound according to any one of claims 1 to 30;

(ii) one or more structural lipids,

(iii) one or more stabilizing agents; and

(iv) optionally, a payload.

50. The composition according to claim 48, comprising:

(i) a compound according to any one of claims 1 to 30;

(ii) one or more structural lipids,

(iii) one or more stabilizing agents;

(iv) one or more transfection enhancing agents; and

(v) optionally, a payload.

51. A composition comprising:

(i) one or more compounds according to any one of claims 1 to 30; and

(ii) a payload.

52. The composition according to any one of claims 48 to 51, wherein the one or more compounds according to claims 1 to 30 is present at 10 to 80 mol% of the composition, excluding any payload, if present.

53. The composition according to any one of claims 48 to 51, wherein the structural lipid is present at 14-50 mol% of the composition, excluding any payload, if present.

54. The composition according to any one of claims 48 to 51, wherein the stabilizing agent is present at 0.1-10 mol% of the composition, excluding any payload, if present.

55. The composition according to any one of claims 48 to 51, further comprising an exosome or a biological material derived or purified from an exosome.

56. The composition of any one of claims 48 to 51, further comprising a polymer.

57. The composition of claim 56, wherein the polymer is selected from the group consisting of: a dense star dendrimer, a PAMAM dendrimer, an NH_3 core dendrimer, an ethylenediamine core dendrimer, a dendrimer of generation 5 or higher, a dendrimer with a substituted group, a dendrimer comprising one or more amino acids, a grafted dendrimer, an activated dendrimer, polyethylenimine, polyethylenimine conjugates, polylysine, polyarginine, polyornithine, histone, and any combination thereof.

58. The composition of any one of claim 56, wherein the polymer is a linear or branched PEI.

59. The composition of any one of claims 48 to 50, and 52 to 58, wherein the stabilizing agent is selected from the group consisting of: a surfactant, a neutral lipid, a polymer-conjugated lipid, polyethylene glycol, a phospholipid, and any combination thereof.

60. The composition of any one of claims 48 to 50, and 52 to 59, wherein the stabilizing agent is a PEG-modified lipid.

61. The composition of any one of claims 48 to 50, and 52 to 59, wherein the one or more transfection enhancing agents comprises a polycationic nucleic acid binding moiety.

62. The composition according to claim 61, wherein the transfection enhancing agent selected from the group consisting of: an endosomal release agent, a cell surface ligand, a nuclear localization agent, a cell-penetrating peptide, a fusogenic peptide, and any combination thereof.

63. The composition of claim 50, wherein the one or more transfection enhancing agents comprises an amphipathic peptide.

64. The composition of any one of claims 48 to 50, and 52 to 63, further comprising a payload.

65. The composition of claim 51 or 64, wherein the payload comprises a nucleic acid.

66. The composition of claim 65, wherein the compound according to claims 1 to 30 comprises a charge N and the nucleic acid molecule comprises a charge P and wherein the combination of the compound according to claims 1 to 30 and the nucleic acid contacting the cell comprises an N/P ratio from about 1 to 20.

67. The composition of claim 65 or 66, wherein the nucleic acid is an RNA.

68. The composition of claim 67, wherein the RNA is mRNA, siRNA, shRNA, self-replicating RNA (srRNA), an o-RNA, self-amplifying RNA, stRNA, trRNA, crRNA, sgRNA, RNAi molecule, an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), or any combination thereof.

69. The composition of claim 65, wherein the nucleic acid is a DNA.

70. The composition of claim 64, wherein the payload further comprises one or more peptides, and optionally a nucleic acid.

71. The composition of claim 70, wherein the peptide is covalently linked to a nucleic acid.

72. The composition of claim 68, wherein the RNA is an mRNA.

73. The composition of claim 72, comprising two or more different mRNAs.

74. The composition of claim 73, wherein the RNA encodes an immunogen.

75. The composition of claim 74, wherein the RNA encodes a cancer antigen.

76. The composition according to any one of claims 48 to 50, wherein the structural lipid is selected from the group consisting of: cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, hopanoids, phytosterols, steroids, and any combination thereof.

77. The composition according to any one of claims 48 to 50, wherein the stabilizing agent comprises one or more phospholipids selected from the group consisting of: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin.

78. The composition according to any one of claims 48 to 50, wherein the ionizable lipid comprises one or more cationic lipid selected from GeneIn™, LipofectAmine™ 2000, LipofectAmine™, Lipofectin®, DMRIE-C, CellFectin® (Invitrogen), Oligofectamine® (Invitrogen), LipofectAce® (Invitrogen), Fugene® (Roche, Basel, Switzerland), Fugene® HD (Roche), Transfectam® (Tranfectam, Promega, Madison, WI), Tfx-10® (Promega), Tfx-20® (Promega), Tfx-50® (Promega), Transfectin™ (BioRad, Hercules, CA), SilentFect™ (Bio-Rad), Effectene® (Qiagen, Valencia, CA), DC-chol (Avanti Polar Lipids), GenePorter® (Gene Therapy Systems, San Diego, CA), DharmaFect 1® (Dharmacon, Lafayette, CO), DharmaFect

2® (Dharmacon), DharmaFect 3® (Dharmacon), DharmaFect 4® (Dharmacon), Escort™ III (Sigma, St. Louis, MO), Escort™ IV (Sigma), DOTMA, DOTAP, DMRIE, DC-Chol, DDAB, DOSPA, DOSPER, DOGS, TMTPS, TMTOS, TMTLS, TMTMS, TMDOS, N-1-dimethyl-N-1-(2,3-diaoleoyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxypropyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalmityloxy-propyl)-2-hydroxypropane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diaoleoyl-oxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diamyristyloxy-propyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, N-1-dimethyl-N-1-(2,3-diapalm-ityloxypropyl)-2-(3-amino-2-hydroxypropyloxy)propane-1,3-diamine, L-spermine-5-carboxyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine, 3,5-(N,N-di-lysyl)-diamino-benzoyl-glycyl-3-(DL-1,2-dipalmitoyl-dimethylaminopropyl-β-hydroxyethylamine), L-Lysine-bis(O,O'-oleoyl-β-hydroxy-ethyl)amidedihydrochloride, L-Lysine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amidedihydrochloride, 1,4-bis[(3-(3-aminopropyl)-alkylamino)-2-hydroxypropyl)-piperazine, L-Lysine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-myristoyl-β-hydroxyethyl)amide dihydrochloride, L-Ornithine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, L-Ornithine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, 1,4,-bis[(3-amino-2-hydroxypropyl)-oleylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxy-propyl)-palmitylamino]-butane-2,3-diol, 1,4,-bis[(3-amino-2-hydroxypropyl)-myristylamino]-butane-2,3-diol, 1,4-bis[(3-oleylamino)propyl]-piperazine, L-Arginine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, bis[(3-(3-aminopropyl)-myristylamino)2-hydroxy-propyl]piperazine, L-Arginine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Serine-bis-(O,O'-oleoyl-β-hydroxyethyl)amide dihydrochloride, 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]piperazine, Glycine-bis-(O,O'-palmitoyl-β-hydroxy-ethyl)amide dihydrochloride, Sarcosine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, L-Histidine-bis-(O,O'-palmitoyl-β-hydroxyethyl)amide dihydrochloride, cholesteryl-3β-carboxyl-amidoethylenetrimethylammonium iodide, 1,4-bis[(3-myristyl-amino)-propyl]-piperazine, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl carboxylate iodide, cholesteryl-3β-carboxyamidoethyleneamine, cholesteryl-3β-oxysuccinamido-ethylene-trimethylammonium iodide, 1-dimethylamino-3-trimethylammonio-DL-2-propyl-cholesteryl-3β-oxysuccinate iodide, 2-[(2-trimethylammonio)-ethylmethyl-amino]ethyl-cholesteryl-3β-

oxysuccinate iodide, 3β[N-(N', N'-dimethylamino-ethane)-carbamoyl]cholesterol, and 3β-[N-(polyethyleneimine)-carbamoyl] cholesterol,1,4-bis[(3-palmitylamino)propyl]piperazine, L-Ornithylglycyl-N-(1-heptadecyloctadecyl)-glycinamide, N^2,N^5-Bis(3-aminopropyl)-L-ornithyl-glycyl-N-(1-heptadecyloctadecyl)-glycinamide, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-alkyl-amino)-2-hydroxypropyl]-piperazine, N2-[N2,N5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dioctadecyl-L-glutamine,N2-[N2,N5-Bis(aminopropyl)-L-ornithyl]-N-N-dioctadecyl-L-α-glutamine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleylamino)2-hydroxypropyl]piperazine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dioctadecyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethyl-ethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioctadecyl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-diolyl-L-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-glutamine,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)-2-hydroxy-propyl]piperazine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dioleyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dioleyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-oleyl-amino)-propyl]piperazine, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dipalmityl-L-glutamine,N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dipalmityl-L-α-glutamine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dipalmityl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)-carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dipalmityl-L-glutaminyl]-L-glutamic acid, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dimyristyl-L-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-glutamine, N^2-[N^2,N^5-Bis(aminopropyl)-L-ornithyl]-N-N-dimyristyl-L-α-asparagine, 1,4-bis[(3-(3-amino-2-hydroxy-propyl)-palmitylamino)-2-hydroxypropyl]-piperazine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethyl-ethoxy)carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dimyristyl-L-glutaminyl]-L-glutamic acid, 1,4-bis[(3-(3-aminopropyl)-myristyl-amino)-propyl]piperazine, N^2-[N^2,N^5-Bis(3-aminopropyl)-L-ornithyl]-N,N-dilaureyl-L-glutamine, N^2-[N^2,N^5-Bis(amino-propyl)-L-ornithyl]-N-N-dilaureyl-L-α-glutamine, N^2-[N^2,N^5–Bis-(amino-propyl)-L-ornithyl]-N-N-dilaureyl-L-α-asparagine, N-[N^2-[N^2,N^5-Bis[(1,1-dimethylethoxy)-carbonyl]-N^2,N^5-bis[3-[(1,1-dimethylethoxy)carbonyl]aminopropyl]-L-ornithyl-N-N-dilaureyl-L-glutaminyl]-L-glutamic acid, 3-[N',N''-bis(2-tertbutyloxycarbonyl-amino-ethyl)guanidino]-N,N-dioctadec-9-enylpropionamide, 3-[N',N''-bis(2-tertbutyloxy-carbonylamino-

ethyl)guanidino]-N,N-dipalmitylpropionamide, 3-[N',N"-bis(2-tertbutyl-oxycarbonyl-aminoethyl)guanidino]-N,N-dimyristylpropionamide, 1,4-bis[(3-(3-amino-propyl)-palmityl-amino)propyl]piperazine, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-oleyl-amino)propyl]piperazine, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-diolylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-dipalmitylaminopropane, N,N-(2-hydroxy-3-aminopropyl)-N-2-hydroxypropyl-3-N,N-dimyristylaminopropane, 1,4-bis[(3-(3-amino-2-hydoxypropyl)-myristylamino)-propyl]piperazine, [(3-aminopropyl)-bis-(2-tetradecyl-oxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-oleyloxyethyl)]methyl ammonium bromide, [(3-aminopropyl)-bis-(2-palmityloxyethyl)]methyl ammonium bromide, oleoyl-2-hydroxy-3-N,N-dimethyamino propane, 2-didecanoyl-1-N,N-dimethylaminopropane, palmitoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dipalmitoyl-1-N,N-dimethylamino-propane, myristoyl-2-hydroxy-3-N,N-dimethyamino propane, 1,2-dimyristoyl-1-N,N-dimethylaminopropane, (3-amino-propyl)-›4-(3-amino-propylamino)-4-tetradecyl-carbamoyl-butylcarbamic acid cholestryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino-4-carbamoylbutylcarbamic acid cholestryl ester, (3-Amino-propyl)-›4-(3-amino-propylamino)-4-(2-dimethylamino-ethylcarbamoy-l)-butylcarbamic acid cholesteryl ester, Spermine-5-carboxyglycine (N'-stearyl-N'-oleyl) amide tetratrifluoro-acetic acid salt, Spermine-5-carboxyglycine (N'-stearyl-N'-elaidyl) amide tetratri-fluoroacetic acid salt, Agmatinyl carboxycholesterol acetic acid salt, Spermine-5-carboxy-β-alanine cholesteryl ester tetratrifluoroacetic acid salt, 2,6-Diaminohexanoeyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, 2,4-Diaminobutyroyl β-alanine cholesteryl ester bistrifluoroacetic acid salt, N,N-Bis (3-aminopropyl)-3-aminopropionyl β-alanine cholesteryl ester tristrifluoroacetic acid salt, [N,N-Bis(2-hydroxyethyl)-2-aminoethyl]aminocarboxy cholesteryl ester, Stearyl carnitine ester, Palmityl carnitine ester, Myristyl carnitine ester, Stearyl stearoyl carnitine ester chloride salt, L-Stearyl Stearoyl Carnitine Ester, Stearyl oleoyl carnitine ester chloride, Palmityl palmitoyl carnitine ester chloride, Myristyl myristoyl carnitine ester chloride, L-Myristyl myristoyl carnitine ester chloride, 1,4-bis[(3-(3-amino-2-hydroxypropyl)-palmityl-amino)propyl]-piperazine, N-(3-aminopropyl)-N,N'-bis-(dodecyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N,N'-bis-(myristyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-dodecyloxyethyl)-

piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-oleyloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-palmityloxyethyl)-piperazinium bromide, N-(3-aminopropyl)-N'-methyl-N,N'-(bis-2-myristyloxyethyl)-piperazinium bromide, 1,4-bis[(3-(3-aminopropyl)-oleylamino)-2-hydroxy-propyl]piperazine, 1,4-bis[(3-(3-aminopropyl)-myristylamino)-2-hydroxy-propyl]piperazine, or 1,4-bis[(3-(3-aminopropyl)-palmitylamino)-2-hydroxypropyl]-piperazine, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-aminopropyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-amino-propyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(2-hydroxy-3-aminopropyl)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diamino-butane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(3-amino-propyl)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(5-carboxamido-spermine)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(5-carboxamidospermine)-diaminobutane, 2,3-dimyrist-oleoyloxy-1,4-N,N'-dimethyl-N,N'-di(5-caqrboxamidospermine)-diaminobutane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diaminobutane, 2,3-dipalmitoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(lysyl)-diamino-butane, 2,3-dioleyloxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dipalmitoleoyl-oxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dimyristoleoyloxy-1,4-N,N'-dimethyl-N,N'-di(histidyl)-diaminobutane, 2,3-dioleyloxy-N,N'-dimethyl-1,4 -diaminobutane, 2,3-dipalmitoleoyloxy-N,N'-dimethyl-1,4-diaminobutane, 2,3-dimyrist-oleoyloxy-N,N'-dimethyl-1,4-diaminobutane; PAMAM dendrimers, NH3 core dendrimers, ethylenediamine core dendrimers, polyethylenimine, and polyethylenimine conjugates.

79. The composition of claim 60, wherein the one or more PEG-modified lipids is selected from the group consisting of: a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-ceramide conjugate, a PEG-modified dialkylamine, a PEG-modified 1,2-diacyloxypropan-3-amine, and any combination thereof.

80. The composition of claim 60, wherein the one or more PEG-modified lipids is selected from the group consisting of: PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DSPE, and any combination thereof.

81. The composition according to any one of claims 48 to 50, further comprising a transfection enhancing agent selected from the group consisting of an endosomal release agent, a cell surface ligand, a nuclear localization agent, a cell-penetrating peptide, a fusogenic peptide, and any combination thereof.

82. A method of delivering a payload to a cell, comprising:

(i) providing a composition according to any one of claims 48 to 81;

(ii) providing a cell; and

(iii) contacting the cell with the composition.

83. A method for delivering a composition to a subject, comprising: administering the composition according to any one of claims 48 to 81 to the subject.

84. The method of claim 82, wherein the contacting the cell is *in vitro*.

85. The method of claim 82, wherein the contacting the cell is *ex vivo*.

86. The method of claim 82, wherein the contacting the cell is *in vivo*.

87. The method of claim 82, wherein the cell is a eukaryotic cell.

88. The method of claim 87, wherein the eukaryotic cell is a mammalian cell.

89. The method of claim 83, wherein the administration is systemic.

90. The method of claim 83, wherein the administration is selected from the group consisting of: subcutaneous administration, intramuscular administration, intranasal administration, intra-tumoral administration, administration to the brain, administration to the spinal cord, administration to the eye, administration to the lymph node of a subject, and any combination thereof.

91. A kit comprising:

(i) one or more compounds according to claims 1 to 30, and

(ii) one or more of a structural lipid, an ionizable lipid, and a stabilizing agent.

92. The kit according to claim 91, comprising:

(i) a compound according to any one of claims 1 to 30;

(ii) one or more structural lipids,

(iii) one or more stabilizing agents; and

(iv) optionally, a payload.

93. The kit according to claim 91, comprising:

(i) a compound according to any one of claims 1 to 30;

(ii) one or more structural lipids,

(iii) one or more stabilizing agents;

(iv) one or more fusion agent; and

(v) optionally, a payload.

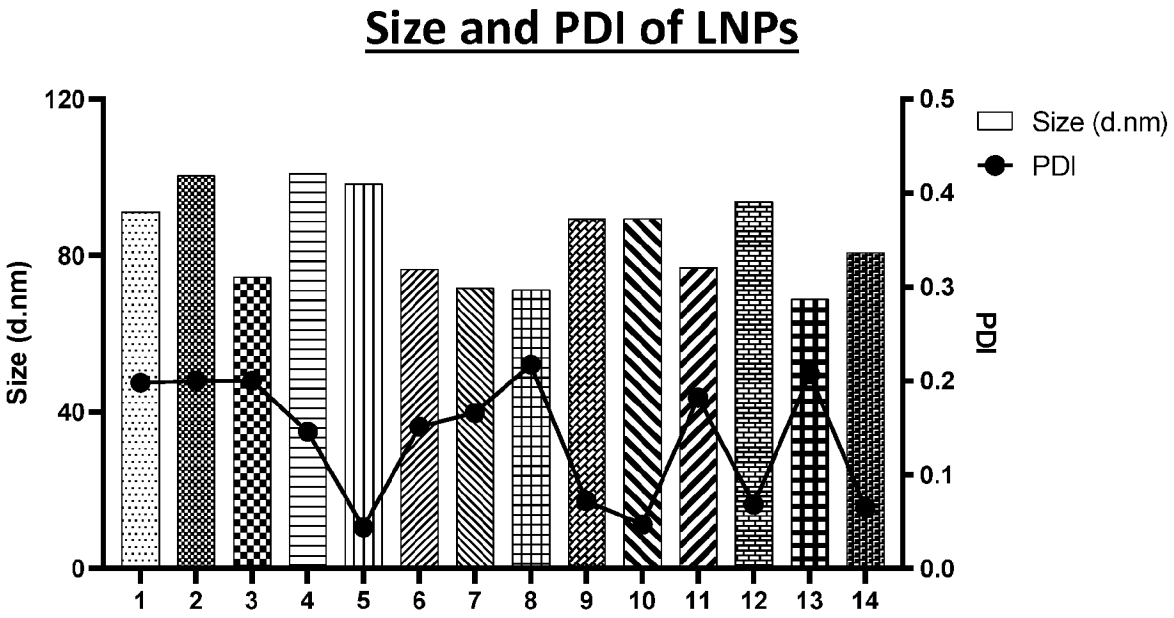

Size and PDI of LNPs
120
80
40
0
Size (d.nm)
0.5
0.4
0.3
0.2
0.1
0.0
PDI
Size (d.nm)
PDI
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 1

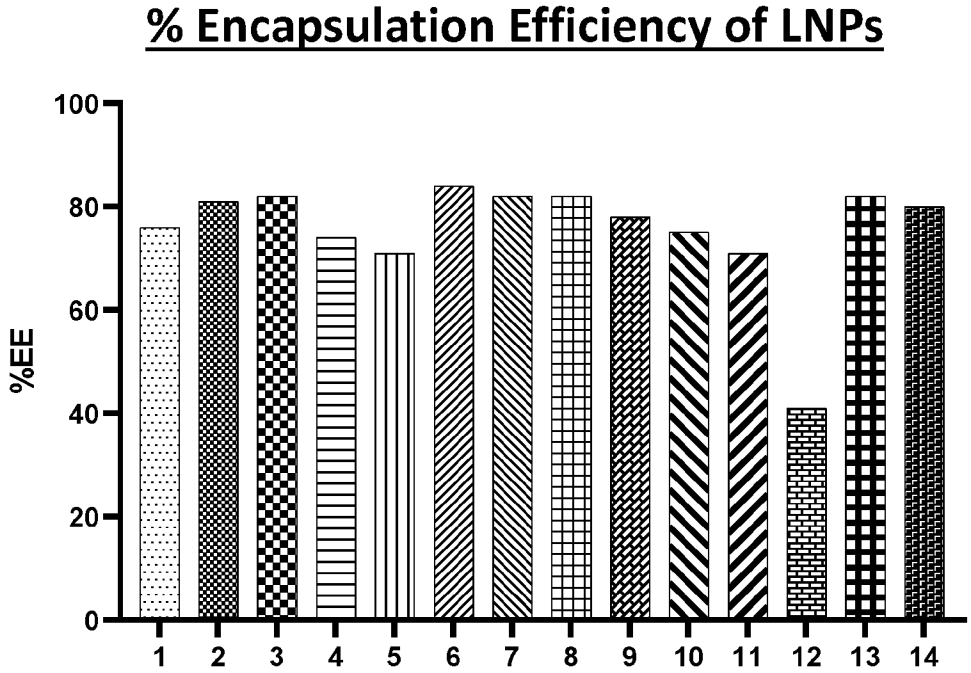

% Encapsulation Efficiency of LNPs
100
80
60
40
20
0
%EE
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 2

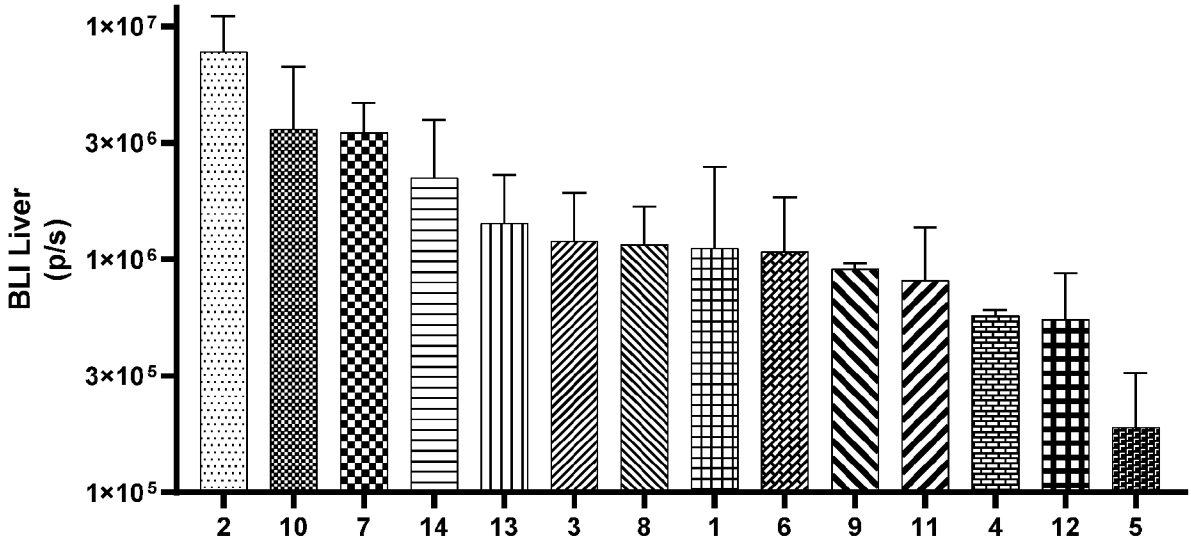

BLI Liver (p/s)
1×10⁷
3×10⁶
1×10⁶
3×10⁵
1×10⁵
2 10 7 14 13 3 8 1 6 9 11 4 12 5

Fig. 3

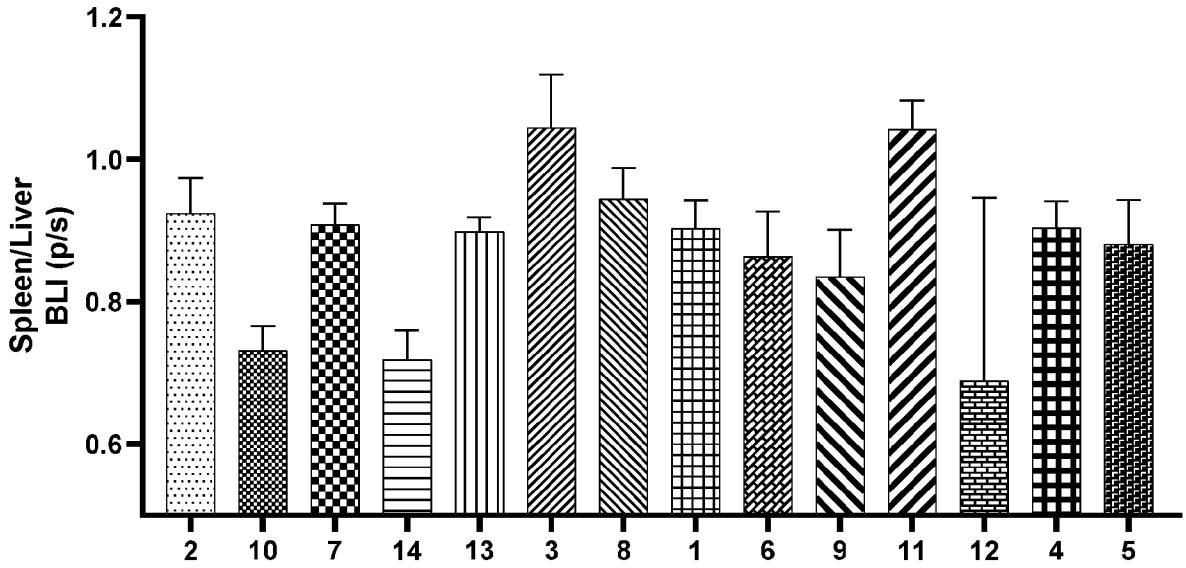

Spleen/Liver BLI (p/s)
1.2
1.0
0.8
0.6
2 10 7 14 13 3 8 1 6 9 11 12 4 5

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/071670

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D265/30 C07D279/12 C07D295/145 A61K48/00
ADD.

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)
C07D 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)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/089239 A1 (ALNYLAM PHARMACEUTICALS INC [US]) 12 June 2014 (2014-06-12) table starting from 118; morpholine compounds on p.120-122, 124-130, 135,138; paragraph [0006] - paragraph [0008] -----	1,2,4,5, 9,12,14, 20-23, 25,27, 31-93
X	WO 2021/030701 A1 (ACUITAS THERAPEUTICS INC [CA]) 18 February 2021 (2021-02-18) morpholine compound on p.102-199 ----- -/--	1,2,4,5, 9,12,14, 20-23, 25,27, 31-93

[X] Further documents are listed in the continuation of Box C.

[X] 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

"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
14 November 2023	20/11/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schuemacher, Anne

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/086354 A1 (ALNYLAM PHARMACEUTICALS INC [US]) 13 June 2013 (2013-06-13) page 56 - page 76	1,2,4,5, 9,12, 20-23, 25,27, 31-93
X	WO 2017/049245 A2 (MODERNATX INC [US]) 23 March 2017 (2017-03-23) paragraph [00488]; claim 148	1,2,12, 20-22, 27,31-93
X	WO 2018/170306 A1 (MODERNATX INC [US]) 20 September 2018 (2018-09-20) paragraph [00214]; claim 184; compound 103	1,2,12, 20-22, 27,31-93
X	AHMED GIASUDDIN ET AL: "Enamine chemistry. Part 25. Preparation and carbon-13 nuclear magnetic resonance spectra of N-alkyl-morpholines and -pyrrolidines. Comparison with the carbon-13 spectra of the corresponding acyclic enamines", JOURNAL OF THE CHEMICAL SOCIETY , PERKIN TRANSACTIONS 2, CHEMICAL SOCIETY , LETCHWORTH, GB, no. 4, 1 January 1978 (1978-01-01), pages 372-376, XP002173068, ISSN: 1472-779X, DOI: 10.1039/P29780000372 table 1	1-4,10
X	Anhua Ying ET AL: "Ionic tagged DABCO grafted on magnetic nanoparticles: a water-compatible catalyst for aqueous aza-Michael addition of amines to [alpha],[beta]-unsaturated amides", Catalysis Science & Technology, 1 January 2014 (2014-01-01), pages 1-27, XP055698718, Retrieved from the Internet: URL:https://pubs.rsc.org/-/content/getauthorversionpdf/C4CY00232F [retrieved on 2020-05-27] entries 6, 9,11,17; table 2	1,6,10

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/071670

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GARIFZYANOV A R ET AL: "Membrane Transport of Inorganic Acids with [alpha]-Aminophosphoryl Compounds", RUSSIAN JOURNAL OF GENERAL CHEMISTRY, NAUKA/INTERPERIODICA, MO, vol. 75, no. 4, 1 April 2005 (2005-04-01), pages 537-540, XP019301056, ISSN: 1608-3350 compound III	1,7,10
X,P	WO 2022/251665 A1 (RENAGADE THERAPEUTICS MAN INC [US]; JAYARAMAN MUTHUSAMY [US]) 1 December 2022 (2022-12-01) morpholine compound with X=-N- on p.46;; paragraph [0417]	1,2,4,5, 9,12, 20-23, 25,27, 31-93

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/071670

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2023/071670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014089239 A1	12-06-2014	AU 2013355258 A1	11-06-2015
		CA 2891911 A1	12-06-2014
		US 2015306039 A1	29-10-2015
		US 2017233734 A1	17-08-2017
		WO 2014089239 A1	12-06-2014
WO 2021030701 A1	18-02-2021	AU 2020328596 A1	31-03-2022
		BR 112022002708 A2	31-05-2022
		CA 3150458 A1	18-02-2021
		CL 2022000351 A1	03-02-2023
		CN 114901253 A	12-08-2022
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