significant challenges to human health. Mycobacteria, for example, is a genus of bacteria responsible for tuberculosis (TB). According to the World Health Organization, worldwide, TB is one of the top 10 causes of death and the leading cause of death from a single infectious agent. Despite current best efforts, there have been significant challenges in the development of effective vaccines for the prevention of many infectious diseases. New efforts in the identification of individual or combinations of antigenic peptides has helped improved the efficiency of vaccines. Nonetheless, significant opportunities remain in the engineering of adjuvants to help efficiently deliver and present these antigenic sequences to professional antigen presenting cells, like dendritic cells. mRNA coding for antigenic peptides or proteins combined with ionizable cationic lipid nanoparticles represent a particularly promising strategy in the development of a vaccine. There is a need for safe and effective therapies comprising SNALP pharmaceutical compositions for delivery of mRNA for treatment and prevention of various diseases, including vaccine compositions.

SUMMARY

In the present disclosure, ionizable cationic lipids (ICLs) are provided. Cationic lipids are engineered with improved stability to oxidative degradation while in storage, while retaining high transfection activity or potency in cells. Aspects of the disclosure are based in part on the discovery that the undesired oxidation and/or degradation of ionizable lipids with polyene chains can be improved by including two or more methylene groups between a pair of alkynyl double bonds. Lipids disclosed herein comprise at least two carbon-carbon double bonds (olefins) spaced with at least two methylene or substituted methylene groups, wherein the substituted methylene is -$C(R_1)(R_2)$- wherein R_1 and R_2 are independently H, alkyl, or halogen. Lipids disclosed herein include two symmetrical polyene hydrocarbon chains each having two carbon-carbon double bonds (olefins) on either side of two, three or four methylene groups. The olefins in the lipid tails separated by at least two methylene groups renders the compounds described herein considerably less susceptible to oxidation compared to compounds separated by one methylene group, for example DLin-MC3-DMA, considered the gold standard in ionizable cationic lipid design and which was reported to have stability issues. In some examples, the compounds provided herein have greater than 30 %, greater than 50 %, greater than 75 %, greater than 90 %, and greater than 95 % reduction in oxidation byproducts when compared to the control LNP. In some examples, the compounds provided herein have greater than 30 %, greater than 50 %, greater than 75 %,

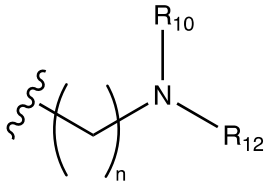
greater than 90 %, and greater than 95 % reduction in oxidation byproducts when compared to the control LNP containing the DLin-KC2-DMA lipid.

In the present disclosure, ionizable cationic lipid compositions are provided. In some examples, the ionizable cationic lipid can comprise two polyene hydrocarbon chains, each including one or two alkenyl double bond moieties. In some examples, the ionizable cationic lipid can comprise two polyene hydrocarbon chains, each including two or more methylene groups between two alkenyl double bond moieties. In some examples, the ionizable cationic lipid can contain two C_{16} or C_{18} polyene hydrocarbon chains.

In the present disclosure, liposomal compositions are provided comprising an ionizable cationic lipid having a pair of linear polyene C_{16} or C_{18} hydrocarbon chains each comprising an unsaturated linear ethylene, n-propylene or n-butylene between two adjacent unsaturated alkynyl double bonds in each polyene hydrocarbon chain. In some examples, a liposomal composition can comprise an ionizable lipid having a chemical structure consisting of a pair of 16 or 18 carbon linear polyunsaturated lipid tails covalently bound to a head group comprising a dialkyl amino group having a pKa of between 6 and 7; wherein the head group comprises a heterocyclyl or alkyl portion covalently bound to the dialkyl amino group and optionally further comprising a phosphate group; and wherein each polyunsaturated lipid tail is unsaturated except for at least two olefins separated by at least two methylene groups along the length of the lipid tail, and each lipid tail optionally comprises a single acyl group at the end covalently bound to the head group. In another aspect, the present disclosure provides a lipid nanoparticle (LNP) composition comprising an ionizable lipid having a chemical structure consisting of a pair of linear polyunsaturated lipid tails covalently bound to a head group, the head group comprising a dialkyl amino group; the head group comprising a heterocyclyl or alkyl portion covalently bound to the dialkyl amino group and optionally further comprising a phosphate group; each polyunsaturated lipid tail being unsaturated except for at least two olefins separated by at least two methylene groups along the length of the lipid tail, and optionally comprising a single acyl group at the end of the lipid tail covalently bound to the head group.

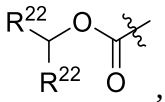
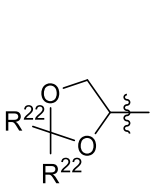
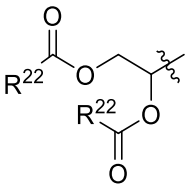
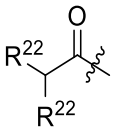
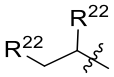
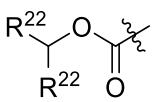
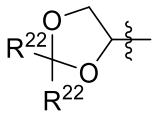
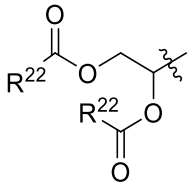
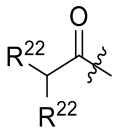
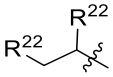
In some examples, each lipid tail is identical, and each lipid tail has a total of two olefins separated only by an unsubstituted ethylene, n-propyl, or n-butyl. In some examples, each lipid tail further comprises an acyl group joined to an oxygen of the headgroup to form an ester.

In some examples, the dialkyl amino portion of the head group of the ionizable cationic lipid has a dialkyl amino chemical structure of Formula (IV-A)

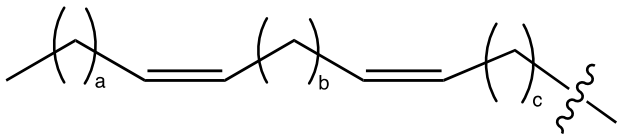


Formula IV-A,

wherein n is 2, 3 or 4 in Formula (IV-A); and R_{10} and R_{12} in Formula (IV-A) are each independently selected from an alkyl group selected from the group consisting of: methyl, ethyl, and n-propyl, wherein the alkyl in R_{10} and R_{12} is optionally substituted with one or more hydroxyl. In some examples, R_{10} and R_{12} in Formula (IV-A) are each independently methyl, ethyl, $–(CH_2)(CH_2)OH$, or $–(CH_2)_2(CH_2)OH$.

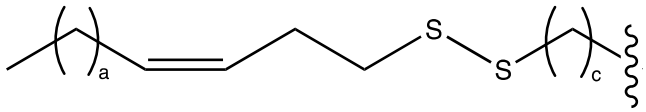
In some examples, an ionizable cationic lipid comprises a chemical structure selected from the group consisting of: , , , , and ; wherein R^{22} is the first end of the lipid tail and  indicates attachment of the head group to the dialkyl amino chemical structure of the head group. In some examples, an ionizable cationic lipid comprising the chemical sub-structure of Formula (IV-A) further comprises an additional chemical structure selected from the group consisting of: , , , , and ; wherein R^{22} is the first end of the lipid tail and  indicates attachment of the head group to the dialkyl amino chemical structure of the head group.

In some examples, an ionizable cationic lipid further comprises a pair of lipid tails attached to the head group, wherein each lipid tail comprises a hydrocarbon chain having the chemical structure of Formula A or Formula B:



Formula A

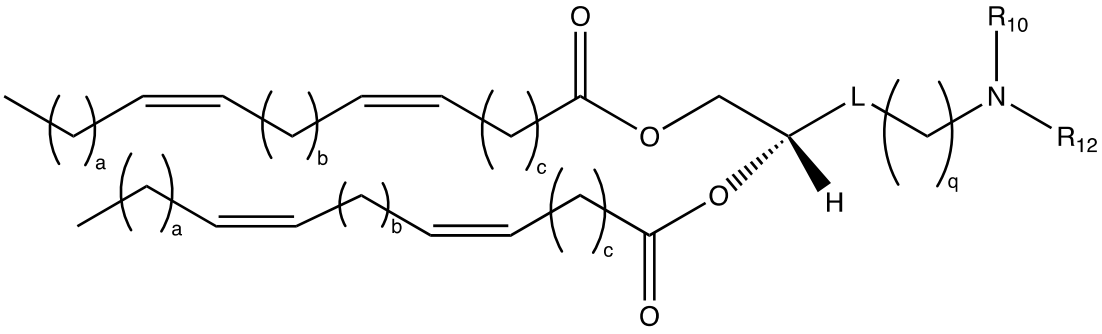
wherein a in Formula A is 1, 2, 3 or 4; b is 2, 3 or 4; and c in Formula A is 3, 4, 5, 6, or7; or



Formula B

wherein a in Formula B is 5, 6 or 7; and c in Formula B is 3, 4, or 5. In some examples, b is 4 and the sum of a, b and c in Formula A is 10, 11, 12 or 13. In some examples, the ionizable cationic lipid comprises a lipid tail of Formula A or Formula B at R^{22} in the chemical structure shown above. In some examples, the ionizable cationic lipid comprises a lipid tail of Formula A, wherein  in Formula A indicates the position of attachment at R^{22} in the chemical structure shown above. In some examples, the ionizable cationic lipid comprises a lipid tail of Formula B, wherein  in Formula B indicates the position of attachment at R^{22} in the chemical structure shown above.

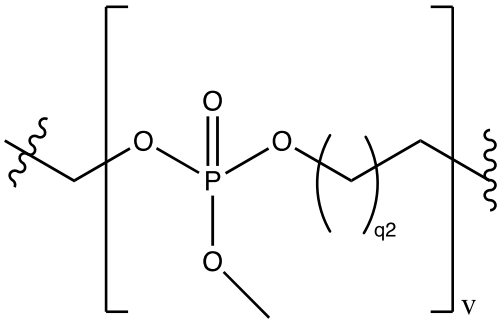
In some examples, the ionizable cationic lipid has a chemical structure of Formula (I-A)



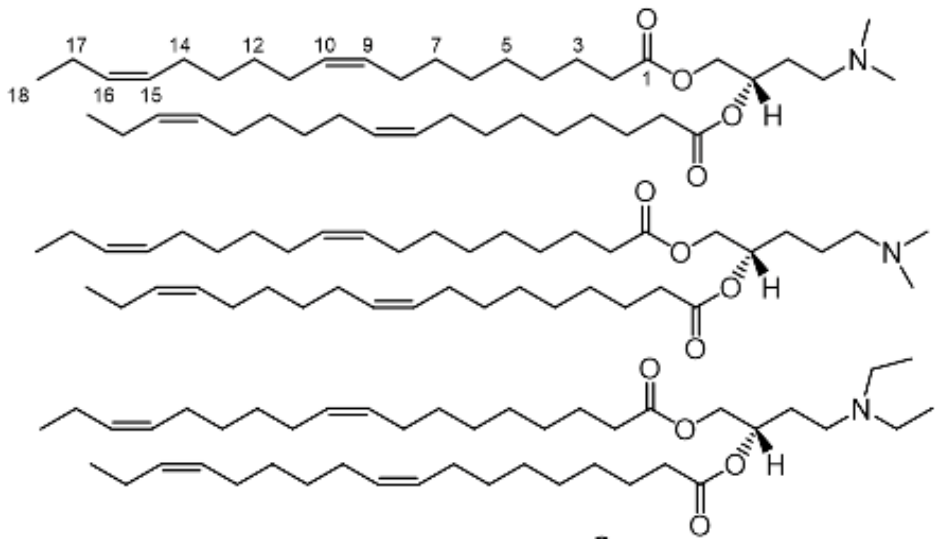
Formula I-A

wherein

a is 1, 2, 3, 4, 5 or 6; b is 2, 3 or 4; c is 3, 4, 5, 6, or 7; and the sum of a, b and c is 10 or 12; q is 1, 2, 3 or 4; each of R_{10} and R_{12} is independently (C_1-C_4)alkyl optionally substituted with one or more hydroxyl; and

L is  , wherein v is 0 or 1; q is 1, 2 or 3 and q2 is 1 or 2. In some examples, q is 1, 2 or 3 when v is 0, and q is 1, 2, 3 or 4 when v is 1 in Formula I-A.

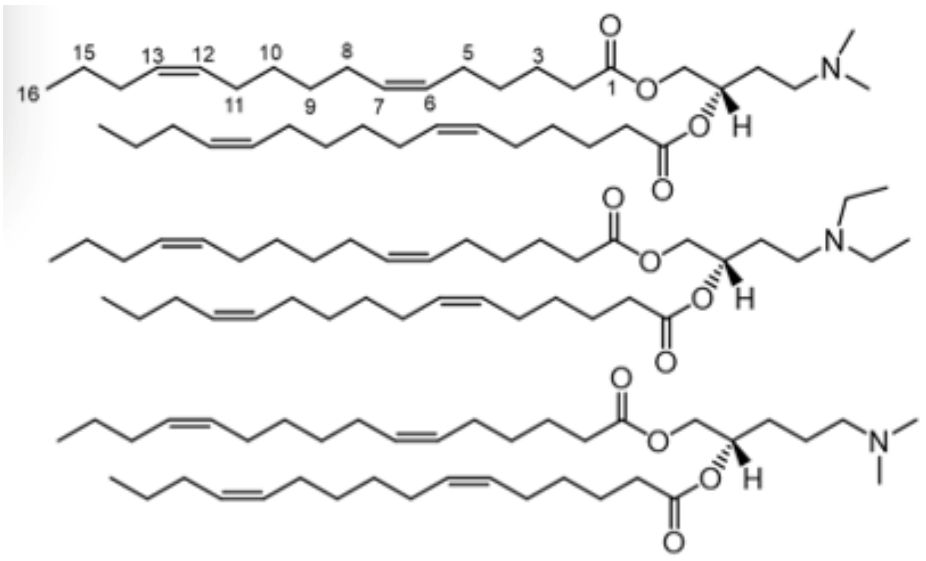
In some examples, v in Formula I-A is 0. In some examples, v in Formula I-A is 0 and q is 1, 2 or 3. In some examples, v in Formula I-A is 0 and q is 1 or 2. In some examples, the ionizable lipid is a cationic lipid selected from the group consisting of Compounds 17-19, and 23-25:



17

18

19

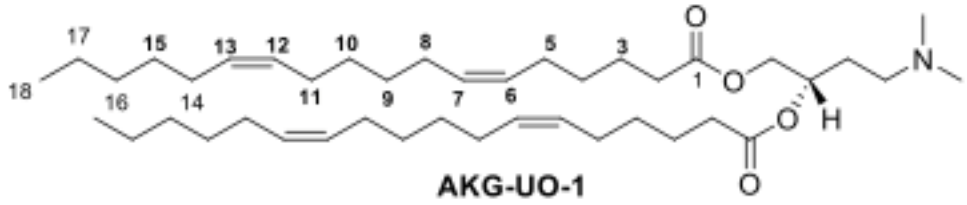


23

24

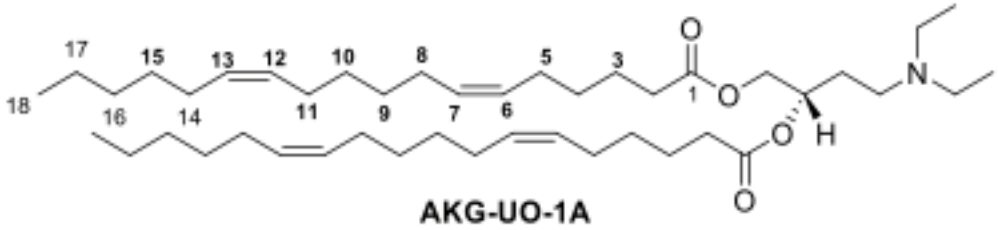
25

In some examples, the ionizable lipid is a cationic lipid selected from the group consisting of: AKG-UO-1, AKG-UO-2, AKG-UO-4, and AKG-UO-5. In some examples, the ionizable lipid is AKG-UO-1:



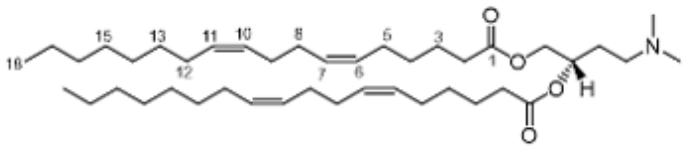
AKG-UO-1

. In some examples, the ionizable lipid is AKG-UO-1A:



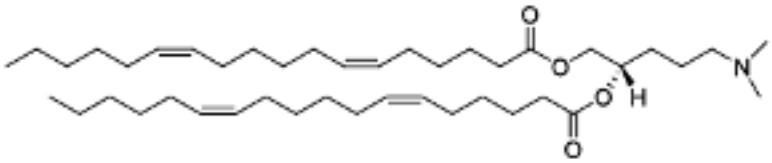
AKG-UO-1A

. In some examples, the ionizable lipid is AKG-UO-1B:



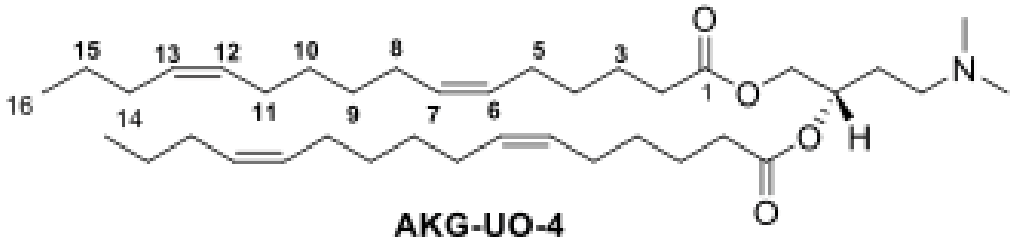
AKG-UO-1B

. In some examples, the ionizable lipid is AKG-UO-2



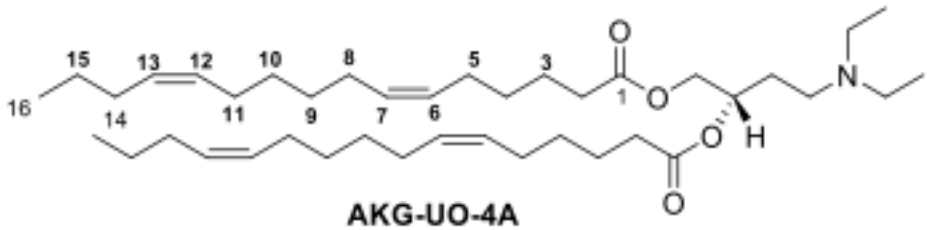
AKG-UO-2

. In some examples, the ionizable lipid is AKG-UO-4:



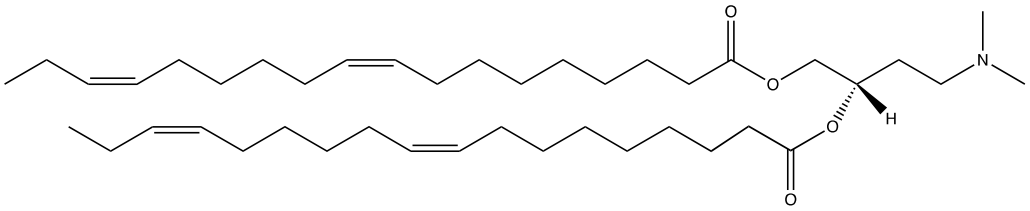
AKG-UO-4

. In some examples, the ionizable lipid is AKG-UO-4A:



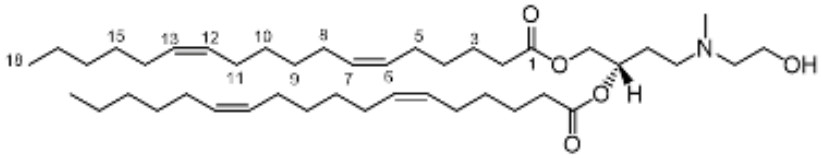
AKG-UO-4A

. In some examples, the ionizable lipid is AKG-UO-5:

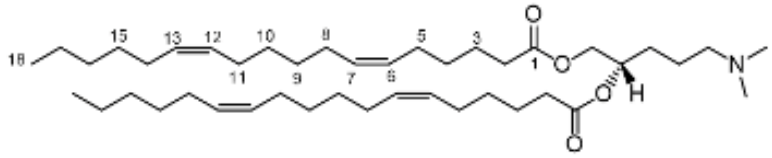


AKG-UO-5

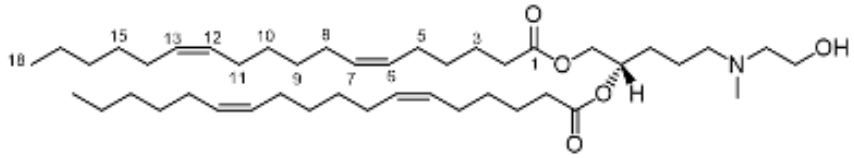
In some examples, the ionizable lipid is AKG-UO-6, AKG-UO-7, AKG-UO-7, AKG-UO-8, AKG-UO-9, or AKG-UO-10:



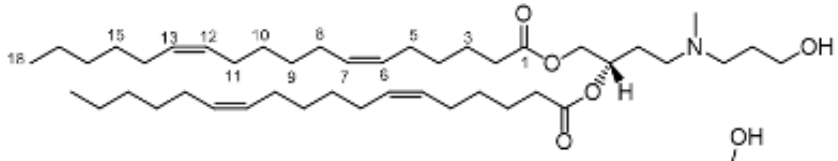
AKG-UO-6



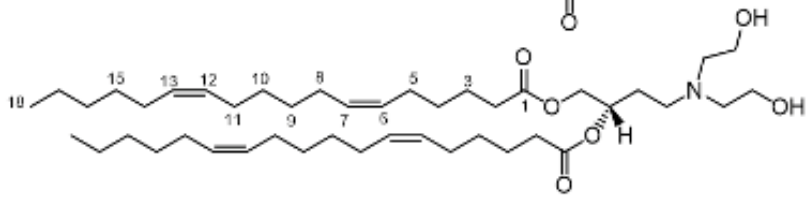
AKG-UO-7



AKG-UO-8



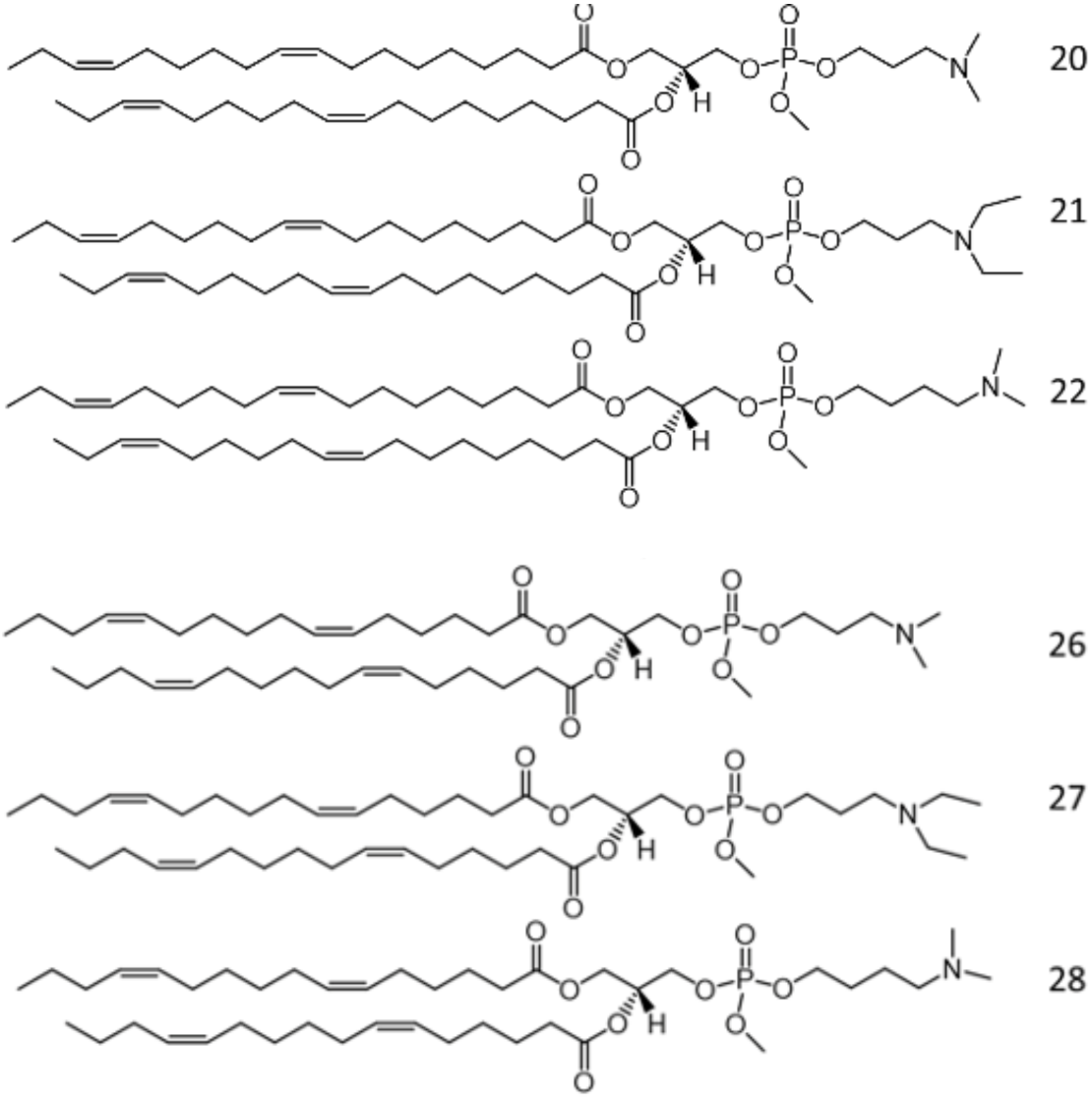
AKG-UO-9



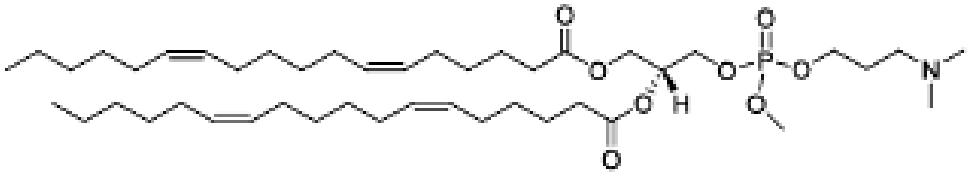
AKG-UO-10

In some examples, the ionizable lipid comprises a head group that includes a methylated phosphate moiety. In some examples, the ionizable lipid has a chemical structure

of Formula I-A wherein v is 1. In some examples, the ionizable lipid has a chemical structure of Formula I-A wherein v is 1 and q is 3 or 4. In some examples, the ionizable lipid is selected from the group consisting of:



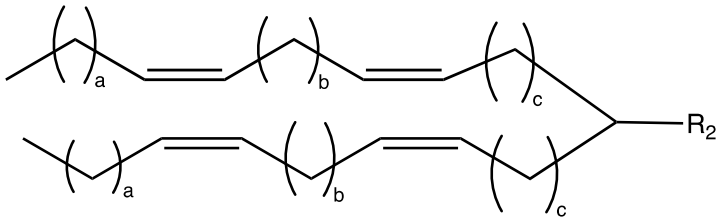
In some examples, the ionizable lipid is AKG-UO-3:



AKG-UO-3

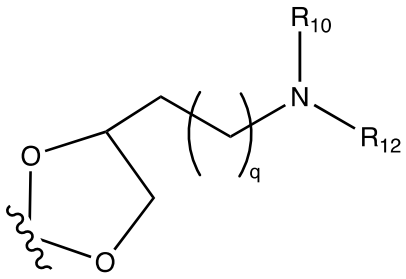
.

In some examples, the ionizable lipid has a chemical structure of Formula II-A:



Formula (II-A)

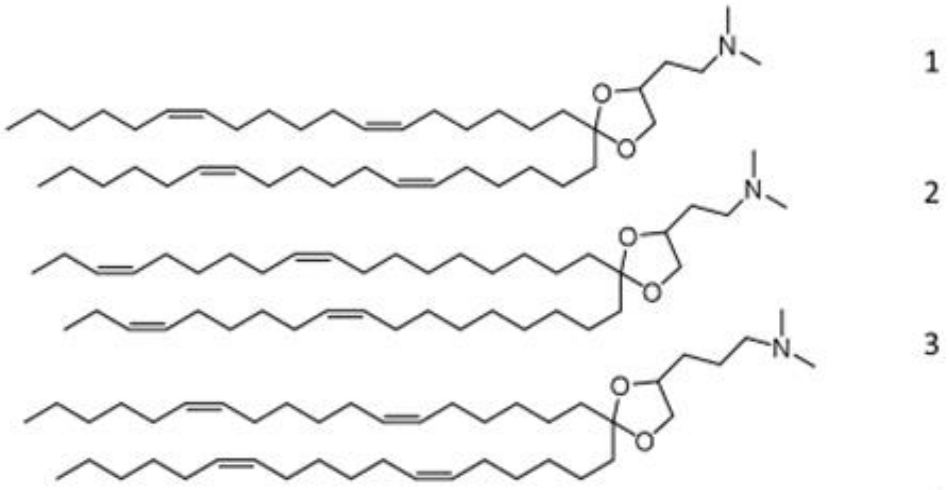
wherein a is 1, 2, 3, 4, 5 or 6; b is 2, 3 or 4; c is 4, 5, 6, 7 or 8;

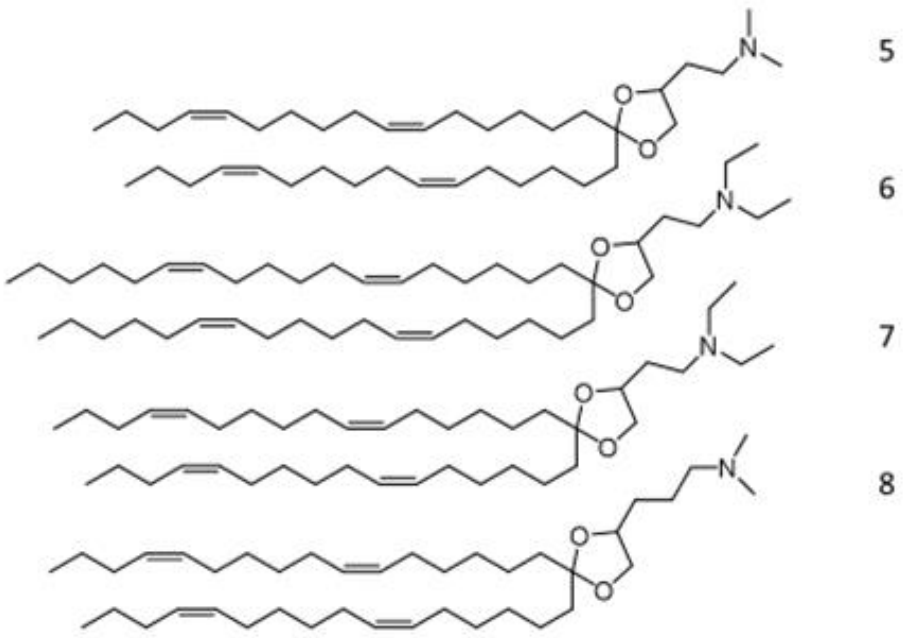
R_2 is  ;

q is 1 or 2; and

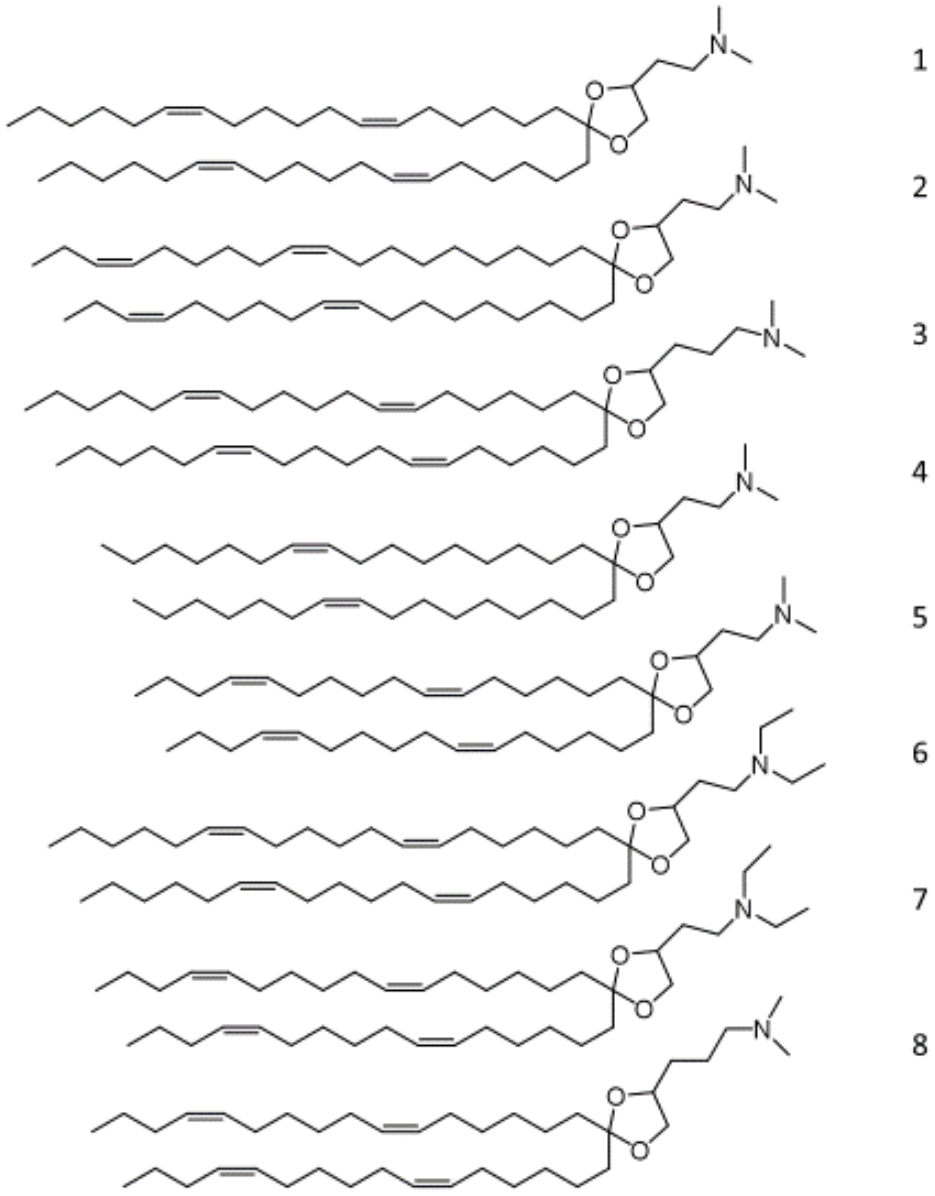
each of R_{10} and R_{12} is independently (C_1-C_4)alkyl optionally substituted with one or more hydroxyl.

In some examples, the ionizable lipid is selected from the group consisting of Compound 1-3 and Compounds 5-8:

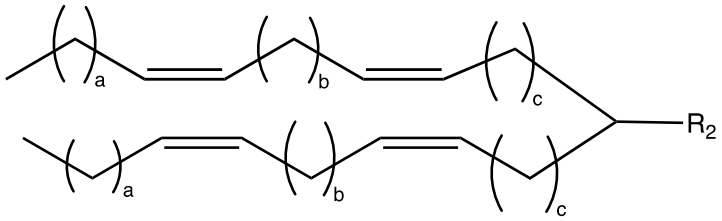




In some examples, the ionizable lipid is selected from the group consisting of Compound 1-8:

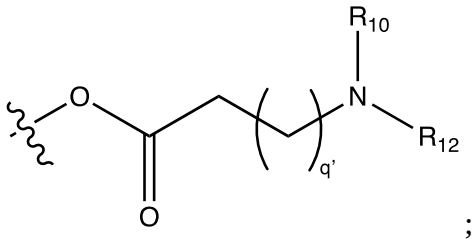


In some examples, the ionizable lipid has a chemical structure of Formula II-A:



Formula (II-A)

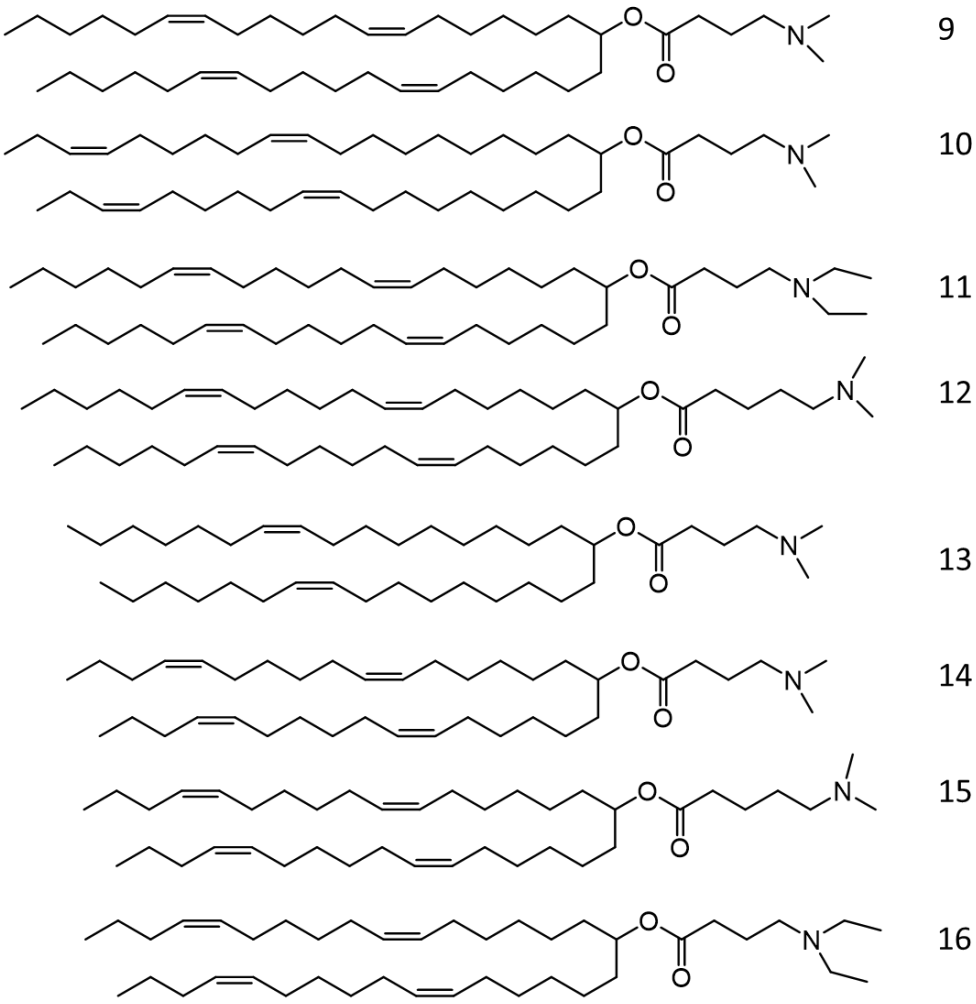
wherein a is 1, 2, 3, 4, 5 or 6; b is 2, 3 or 4; c is 4, 5, 6, 7 or 8;

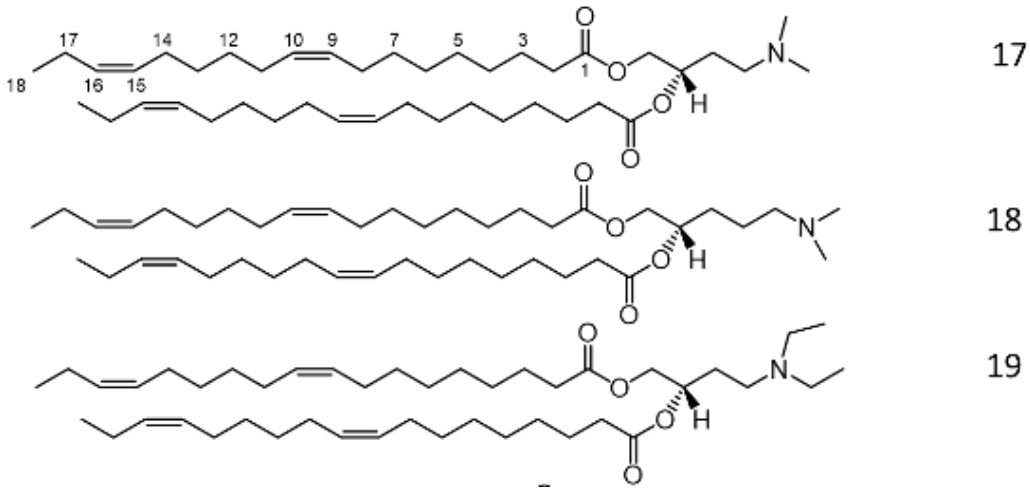
R_2 is  ;

q’ is 1 or 2; and

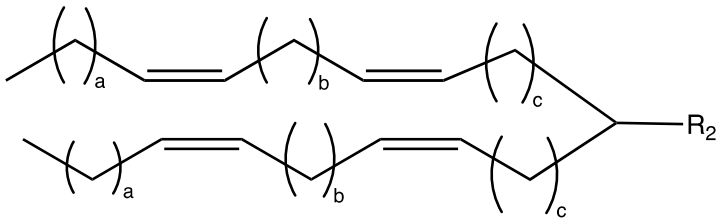
each of R_{10} and R_{12} is independently (C_1-C_4)alkyl optionally substituted with one or more hydroxyl.

In some examples, the ionizable lipid is a compound selected from the group consisting of Compounds 9-19:



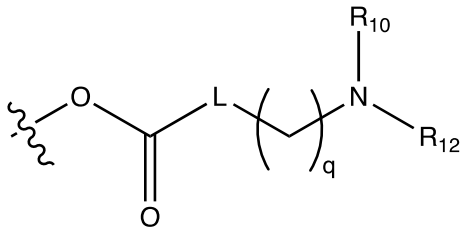


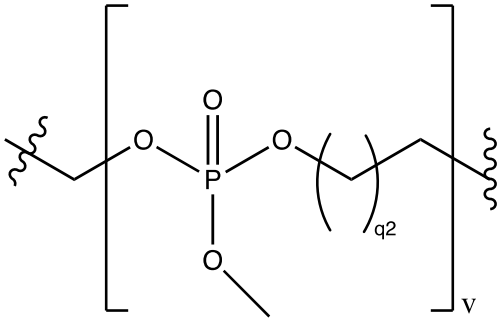
In some examples, the ionizable lipid has a chemical structure of Formula II-A:



Formula (II-A)

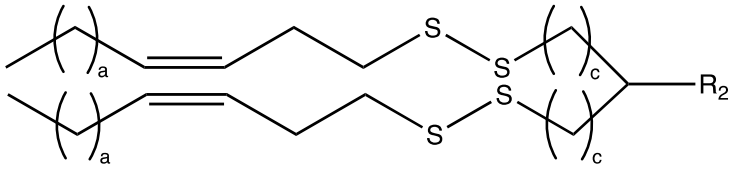
wherein a is 1, 2, 3, 4, 5 or 6; b is 2, 3 or 4; c is 4, 5, 6, 7 or 8;

R_2 is  ;

L is  , wherein v is 0 or 1; q is 1, 2, 3 or 4; and q2 is 1 or 2; and each of R_{10} and R_{12} is independently (C_1-C_4)alkyl optionally substituted with one or more hydroxyl. In some examples, q is 1, 2 or 3 when v is 0 or q is 3 or 4 when v is 1, in Formula II-A.

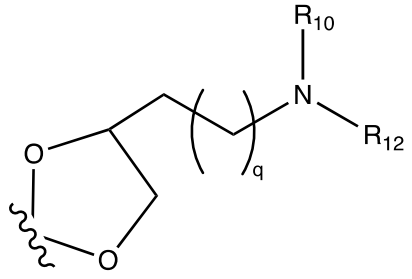
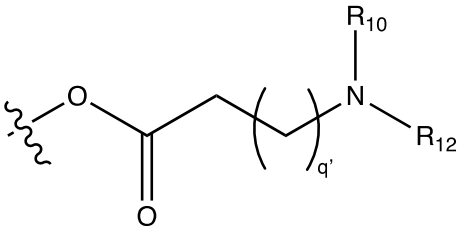
In some examples, the lipids are designed to be biodegradable, thus improving the tolerability of nanoparticles formed with them *in vivo*.

In some examples, the ionizable lipid has a chemical structure of Formula II-B:



Formula (II-B)

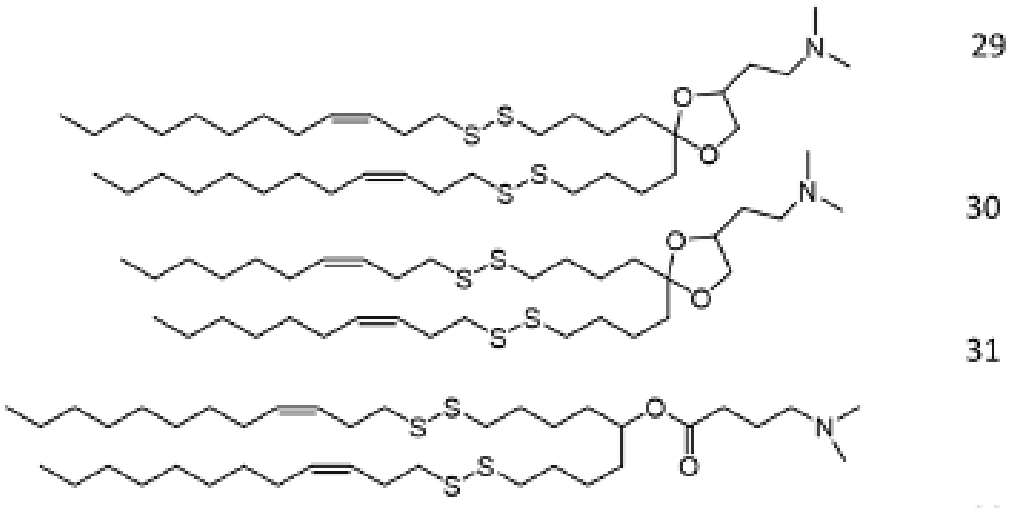
wherein a is 5, 6 or 7; and c is 3, 4, or 5;

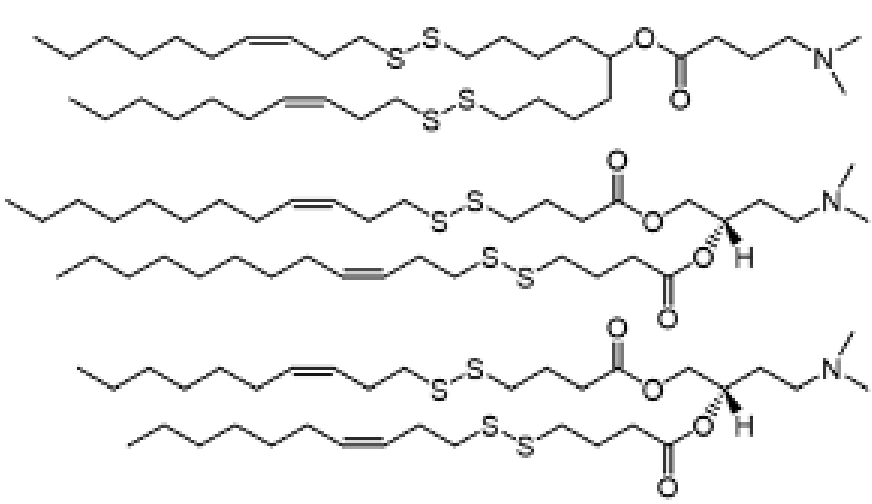
R_2 is  or ;

q and q' are each independently 1 or 2; and

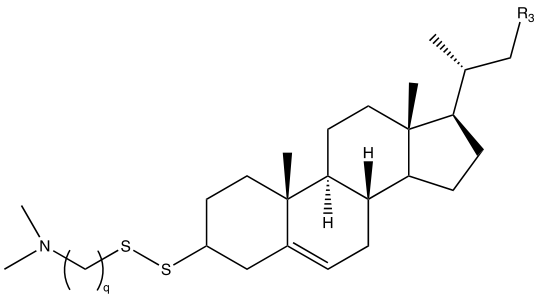
R_{10} and R_{12} are each (C_1-C_4)alkyl optionally substituted with hydroxyl.

In some examples, the ionizable cationic lipid is a compound selected from the group consisting of Compounds 29-34:

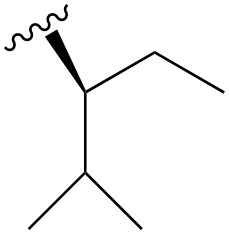
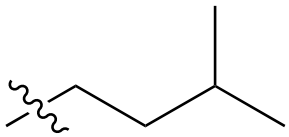


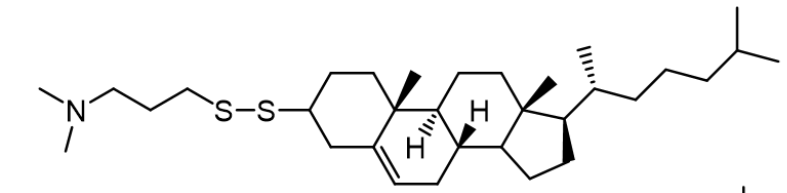


In some examples, the ionizable lipid is a bioreducible cationic lipid. In some examples, the ionizable lipid is a bioreducible cationic lipid comprising a sterol chemical structure. In some examples, the ionizable lipid has a chemical structure of Formula (VI-A):

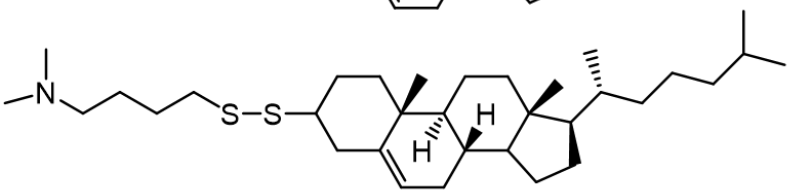


(VI-A)

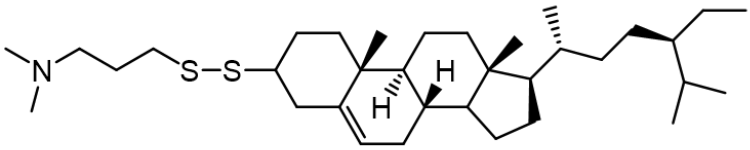
or a pharmaceutically acceptable salt thereof, wherein q is 3 or 4 and R_3 is  or . In some examples, the ionizable cationic lipid is selected from the group consisting of Compounds 35-38:



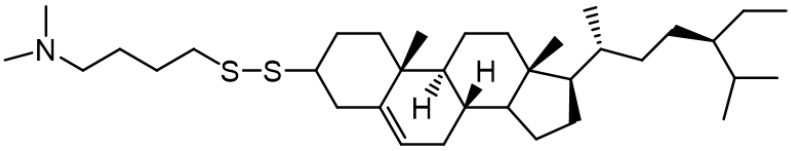
35



36



37



38

In the present disclosure, a lipidic nanoparticle composition comprises lipids and nucleic acids, the lipidic nanoparticles comprising an ionizable lipid of Formula I, II, III, IV or combinations thereof or pharmaceutically acceptable salts thereof. In some examples, a lipidic nanoparticle composition comprises lipids and nucleic acids, the lipidic nanoparticles comprising an ionizable lipid of Formula I-A, II-A, II-B, IV-A, or VI-A or combinations thereof or pharmaceutically acceptable salts thereof. In some examples, a lipidic nanoparticle composition comprises lipids and nucleic acids, the lipidic nanoparticles comprising an ionizable lipid comprising a polyene hydrocarbon chain of Formula A, Formula A′, Formula A″, or Formula B or combinations thereof or pharmaceutically acceptable salts thereof.

The present disclosure also provides for compositions of lipid nanoparticles (LNP) for the delivery of therapeutic nucleic acids to cells. Aspects of the disclosure are based in part on the discovery that LNP compositions combining various ionizable cationic lipids with certain low amounts of a phosphatidyl-L-serine below 20 mol% of the total lipid in the composition (e.g., 2.5-10 mol % of the total lipid in the composition) surprisingly demonstrated significantly enhanced targeting of encapsulated nucleic acids.

In some examples, the LNP compositions comprise: (a) a nucleic acid, (b) an ionizable cationic lipid, (c) a sterol (e.g., cholesterol or a cholesterol derivative, or a phytosterol such as

beta-sitosterol), (d) a phospholipid comprising phosphatidylserine (e.g., a mixture of phosphatidylserine and DSPC), and (e) a conjugated lipid (e.g., PEG-DMG). In one aspect, the present disclosure provides a nucleic acid lipid nanoparticle (LNP) composition comprising: a. a nucleic acid; b. an ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; c. a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition; d. one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising an anionic phospholipid targeting moiety in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and e. optionally further comprising a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition. In one example, the LNP compositions comprise: (a) a nucleic acid, (b) an ionizable cationic lipid, (c) a sterol (e.g., cholesterol or a cholesterol derivative, or a phytosterol such as beta-sitosterol); (d) phospholipids comprising a phosphatidylserine lipid in a total amount of 1-10 mol% (e.g., 2.5-10 mol%, 3-9 mol%, 5.0-7.5 mol%) of the total lipid in the composition, and additional phospholipids (e.g., DSPC), and (e) a conjugated lipid (e.g., PEG-DMG). In one example, the LNP compositions comprise: (a) a nucleic acid, (b) an ionizable cationic lipid, (c) a sterol (e.g., cholesterol or a cholesterol derivative, or a phytosterol such as beta-sitosterol); (d) phospholipids comprising a phosphatidylserine lipid in a total amount of 1-10 mol% (e.g., 2.5-10 mol%, 3-9 mol%, 5.0-7.5 mol%) of the total lipid in the composition, and additional phospholipids (e.g., DSPC), and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-4.5 mol% (e.g., 0.5-2.5 mol%, 1.5 mol%) of the total lipid in the composition. In one example, the LNP compositions comprise: (a) a nucleic acid, (b) an ionizable cationic lipid in a total amount of 40-65 mol% (e.g., 50 mol %) of the total lipid in the composition, (c) a sterol (e.g., cholesterol or a cholesterol derivative, or a phytosterol such as beta-sitosterol) in a total amount of 25-40 mol% (e.g., 38.5 mol%) of the total lipid in the composition; (d) phospholipids comprising a phosphatidylserine lipid in a total amount of 1-10 mol% (e.g., 2.5-10 mol%, 3-9 mol%, 5.0-7.5 mol%) of the total lipid in the composition, and additional phospholipids (e.g., DSPC), and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-4.5 mol% (e.g., 0.5-2.5 mol%, 1.5 mol%) of the total lipid in the composition. In one example, the LNP compositions comprise:

(a) a nucleic acid, (b) an ionizable cationic lipid in a total amount of 40-65 mol% (e.g., 50 mol %) of the total lipid in the composition, (c) a sterol (e.g., cholesterol or a cholesterol derivative, or a phytosterol such as beta-sitosterol) in a total amount of 25-40 mol% (e.g., 38.5 mol%) of the total lipid in the composition; (d) phospholipids in a total amount of 5-25 mol% of the total lipid in the composition, wherein the phospholipids comprise a phosphatidylserine lipid in a total amount of 1-10 mol% (e.g., 2.5-10 mol%, 3-9 mol%, 5.0-7.5 mol%) of the total lipid in the composition, and additional phospholipids (e.g., DSPC) (e.g., 10 mol% of the total lipid in the composition), and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-4.5 mol% (e.g., 0.5-2.5 mol%, 1.5 mol%) of the total lipid in the composition.

In one example, the LNP compositions comprise: (a) a nucleic acid, (b) a ionizable cationic lipid having a pair of linear polyene C_{16} or C_{18} hydrocarbon chains each comprising an unsaturated

linear ethylene, n-propylene or n-butylene between two adjacent unsaturated alkynyl double bonds in each polyene hydrocarbon chain, the ionizable cationic lipid present in the composition in a total amount of 40-65 mol% of the total lipid in the composition, (c) a sterol (e.g., cholesterol) in a total amount of 25-40 mol% of the total lipid in the composition; (d) phospholipids in a total amount of 5-25 mol% of the total lipid in the composition, the phospholipids including a phosphatidylserine lipid (e.g., phosphatidyl-L-serine lipid) in a total amount of 1-10 mol% of the total lipid in the composition, and additional phospholipids (e.g., DSPC in a total amount of 10 mol% of the total lipid in the composition), and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-2.5 mol% of the total lipid in the composition. In one example, the LNP compositions comprise: (a) a mRNA nucleic acid, (b) an ionizable cationic of Formula (I-A), Formula (II-A), or Formula (II-B) when v is 0 in a total amount of 40-65 mol% of the total lipid in the composition, (c) cholesterol in a total amount of 25-40 mol% of the total lipid in the composition; (d) a L-serine phosphatidylserine lipid (e.g., DPPS or DSPS) in a total amount of 1-10 mol% (e.g., 2.5-10 mol%, 3-9 mol%, 5.0-7.5 mol%) of the total lipid in the composition, and DSPC in a total amount of 5-25 mol% of the total lipid in the composition, and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-2.5 mol% of the total lipid in the composition. In one example, the LNP compositions comprise: (a) a mRNA nucleic acid, (b) an ionizable cationic of Formula (I-A) when v is 0 in a total amount of 40-65 mol% of the total lipid in the composition, (c) cholesterol in a total amount of 25-40 mol% of the total lipid in the composition; (d) a L-serine phosphatidylserine lipid (e.g., DPPS or DSPS) in a total amount of 3-9 mol% of the total lipid in the composition, and DSPC in a total amount of 5-25 mol% of the total lipid in the composition, and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-2.5 mol% of the total lipid in the composition.

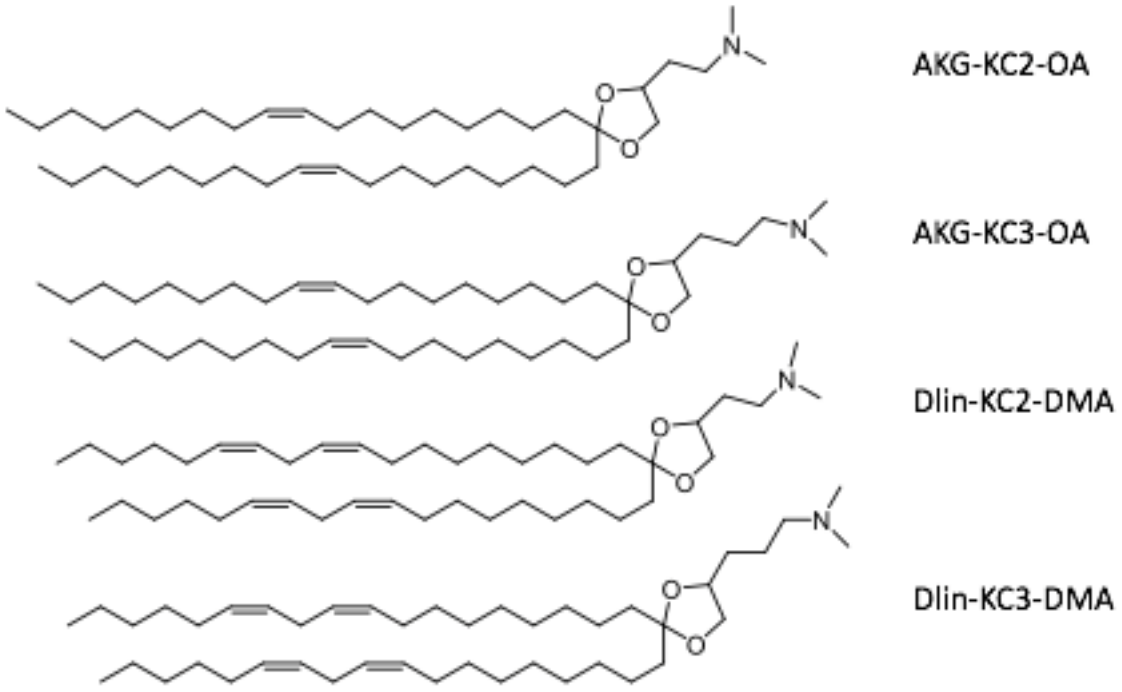
In some examples, a composition comprises : (a) a polyunsaturated ionizable cationic lipid; and (b) a charged phospholipid phosphatidylserine lipid

In some examples, a composition comprises : (a) an ionizable cationic lipid of Formula IV-A; and (b) an anionic phospholipid targeting moiety selected from the group consisting of: DSPS (L-isomer), DPPS (L-isomer), DMPS (L-isomer), DOPS (L-isomer), DSPS (D-isomer), DSPG, DPPG, N-Glu-DSPE, and N-Suc-DSPE. In some examples, a composition comprises: (a) an ionizable cationic lipid of Formula IV-A; and (b) an anionic phospholipid targeting moiety of Formula V-A. In some examples, a composition comprises : (a) an ionizable cationic lipid

of Formula IV-A; and (b) an anionic phospholipid targeting moiety selected from the group consisting of: DSPS (L-isomer), and DPPS (L-isomer).

In some examples, a composition comprises: (a) an ionizable cationic lipid of Formula IV; and (b) an anionic phospholipid targeting moiety of Formula V-A. In some examples, a composition comprises: (a) an ionizable cationic lipid of Formula IV; and (b) an anionic phospholipid targeting moiety selected from the group consisting of: DSPS (L-isomer), DPPS (L-isomer), DMPS (L-isomer), DOPS (L-isomer), DSPS (D-isomer), DSPG, DPPG, N-Glu-DSPE, and N-Suc-DSPE. In some examples, a composition comprises: (a) an ionizable cationic lipid of Formula IV; and (b) an anionic phospholipid targeting moiety of Formula V-A. In some examples, a composition comprises: (a) an ionizable cationic lipid of Formula IV-A; and (b) an anionic phospholipid targeting moiety selected from the group consisting of: DSPS (L-isomer), and DPPS (L-isomer).

In one example, the LNP compositions comprise: (a) an mRNA nucleic acid, (b) an ionizable cationic lipid selected from the group consisting of AKG-KC2-OA, AKG-KC3-OA, Dlin-KC2-DMA and Dlin-KC3-DMA in a total amount of 40-65 mol% of the total lipid in the composition, (c) cholesterol (or a derivative thereof) in a total amount of 25-40 mol% of the total lipid in the composition; (d) a mixture of two or more phospholipids in a total amount of 5-25 mol% of the total lipid in the composition, the phospholipids comprising L-serine phosphatidylserine lipid (e.g., DPPS or DSPS) in a total amount of 3-9 mol% (e.g., 5.0-7.5 mol%) of the total lipid in the composition, and (e) a conjugated lipid (e.g., PEG-DMG) in a total amount of 0.5-2.5 mol% of the total lipid in the composition. In another aspect, the present disclosure provides a nucleic acid lipid nanoparticle (LNP) vaccine composition comprising an ionizable cationic lipid selected from the group consisting of: KC3-OA, Dlin-KC3-DMA, and KC3-O1, wherein the vaccine composition comprises the ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP vaccine composition.



In one example, the nucleic acid lipid nanoparticle (LNP) composition comprises: a nucleic acid, the ionizable cationic lipid AKG-UO-1, and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition. In another aspect, the present disclosure provides a nucleic acid lipid nanoparticle (LNP) composition comprising a Dlin-KC2-DMA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition, in combination with a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition. In some examples, the nucleic acid is mRNA, the PS lipid is (L-Serine) DSPS, (L-Serine) DPPS, or a mixture thereof, and the LNP composition further comprises cholesterol and a second phospholipid selected from the group consisting of: DSPC, DPPC and DOPC. The the LNP composition further comprises 0.5-1.5 mol% PEG-DMG or PEG-DSG, based on the total lipid content in the LNP composition.

In one example, the nucleic acid lipid nanoparticle (LNP) composition comprises: a nucleic acid, an ionizable cationic lipid selected from KC2OA, KC2, KC2-01, ALC-0315, and SM102; and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition. In some examples, the LNP composition has a N/P ratio 3 to 8 (e.g. ratio of 5-7 or 5).

In one example, the nucleic acid lipid nanoparticle (LNP) composition comprises: a nucleic acid, an ionizable cationic lipid selected from AKG-UO-6 and AKG-UO-7; and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition. In some examples, the N/P ratio 3 to 8 (e.g. ratio of 5-7 or 5 or 7).

In one example, the nucleic acid lipid nanoparticle (LNP) vaccine composition comprises: a mRNA nucleic acid with a N/P ratio of 3 to 8; an ALC-0315 ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

In one example, the nucleic acid lipid nanoparticle (LNP) vaccine composition comprises: a mRNA nucleic acid with a N/P ratio of 3 to 8; a Dlin-KC2-DMA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

In one example, the lipid nanoparticle (LNP) vaccine composition comprises: a mRNA nucleic acid with a N/P ratio of 3 to 8; a KC3-OA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

In one example, the nucleic acid lipid nanoparticle (LNP) vaccine composition comprises: a mRNA nucleic acid with a N/P ratio of 3 to 8; an ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition; cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

In another aspect, the disclosure relates to the use of a (L-Serine) PS lipid in a LNP in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition for targeting of the LNP to dendritic cells. In some examples, the LNP comprises mRNA. In some examples, the

LNP further comprises cholesterol. In some examples, the LNP further comprises ICL. In some examples, the LNP further comprises one or more additional phospholipids including DSPC. In some examples, the LNP further comprises a conjugated lipid. In some examples, the LNP comprises a mRNA nucleic acid with a N/P ratio of 3 to 8; an ionizable cationic lipid (ICL) in a total amount of 40-65 mol% of the total lipid content of the LNP composition; cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition; a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and a conjugated lipid in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a depiction of the oxidative degradation mechanisms of lipid esters of linoleic acid containing conjugated multiple unsaturations that are particularly sensitive to oxidation.

FIG. 2 shows the reaction of the reduced c-terminal cysteine of a Fab' antibody fragment with a maleimide terminated-poly(ethylene glycol) 2000 derivatized distearoylphosphatidylethanolamine. R1 and R2 are stearic acid. The final antibody lipopolymer conjugate is an intermediate that is subsequently inserted into the outer lipid layer of lipidic nanoparticle to make it actively targeted.

FIG. 3A. Impact of DSPS inclusion from 0-2.5 mol % on transfection efficiency of dendritic cells (MutuDC1940) using mCherry mRNA LNPs formulated with DLin-KC2-DMA as the ionizable cationic lipid. ICL was kept at 50 mol%, cholesterol at 38.5 mol%, PEG-DMG at 1.5 mol% and the DSPS content varied. Inclusion of DSPS was made by reducing the DSPC content by the same mol% of DSPS that was added. Cells were incubated with each formulation at a concentration of 1 ug mRNA/mL for 24 h. UT sample corresponds to cells where no LNPs were added. Lipofect refers to Lipofectamine treated sample.

FIG. 3B. Impact of DSPS inclusion from 0-7.5 mol % on transfection efficiency of dendritic cells (MutuDC1940) using mCherry mRNA LNPs formulated with DLin-KC2-DMA as the ionizable cationic lipid. ICL was kept at 50 mol%, cholesterol at 38.5 mol%, PEG-DMG at 1.5 mol% and the DSPS content varied. Inclusion of DSPS was made by reducing the DSPC content by the same mol% of DSPS that was added. Cells were incubated with each formulation

CLAIMS

What is claimed is:

1. A nucleic acid lipid nanoparticle (LNP) composition comprising:
    a. a nucleic acid;
    b. an ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
    c. a sterol in a total amount of 25-45 mol% of the total lipid content of the LNP composition;
    d. one or more phospholipids in a total amount of phospholipids of 5-25 mol% of the total lipid content of the LNP composition, and comprising an anionic phospholipid targeting moiety in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition; and
    e. optionally further comprising a conjugated lipid in a total amount of 0.5 – 2.5 mol% of the total lipid content of the LNP composition.
2. The composition of claim 1, wherein the nucleic acid is mRNA,
    optionally wherein the sterol is cholesterol,
    optionally herein the one or more phospholipid consists of: DSPC and a L-serine PS.

3. The composition of claim 2, wherein the LNP composition comprises the PS in a total amount of 5.0-7.5 mol% of the total lipid in the composition,
    optionally wherein the conjugated lipid comprises PEG,
    optionally wherein the conjugated lipid is PEG-DMG,
    optionally wherein the LNP composition comprises the conjugated lipid in a total amount of 0.5-1.5 mol% of the total lipid content of the LNP composition or wherein the LNP composition comprises the conjugated lipid in a total amount of less than 1 mol% of the total lipid content of the LNP composition.
4. The composition of claim 1, wherein:
    a. the nucleic acid is a mRNA,
    b. the ionizable cationic lipid in a total amount of 45-55 mol% of the total lipid content of the LNP composition;

c. a sterol is cholesterol in a total amount of 35-45 mol% of the total lipid content of the LNP composition;
d. the total amount of phospholipid of 7-15 mol% of the total lipid content of the LNP composition;
e. the one or more phospholipids consist of DSPC and the PS lipid is one or more lipids selected from the group consisting of the L-serine configuration of DPPS and DSPS; and
f. the total amount of the PS lipid is 3-9 mol% of the total lipid content of the LNP composition.

5. The composition of claim 4, wherein the composition comprises the PS lipid in a total amount selected from 1.25 mol%, 2.5 mol%, 5 mol%, 7.5 mol%, and 10 mol% of the total lipid content of the LNP composition.
6. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, comprising:
    a. a nucleic acid, wherein the nucleic acid is mRNA;
    b. an ionizable cationic lipid, the ionizable cationic lipid in a total amount of 45-55 mol% of the total lipid content of the LNP composition;
    c. a sterol, wherein the sterol is cholesterol in a total amount of 35-45 mol% of the total lipid content of the LNP composition;
    d. one or more phospholipids, wherein the one or more phospholipids in a total amount of phospholipids of 10 mol% of the total lipid content of the LNP composition, and comprising the anionic phospholipid targeting moiety in a total amount of 3-9 mol% of the total lipid content of the LNP composition; and
    e. a conjugated lipid, the conjugated lipid in a total amount of 0.5 – 1.5 mol% of the total lipid content of the LNP composition,

optionally (i) wherein the anionic phospholipid targeting moiety is selected from the group consisting of: DSPS (L-isomer), DPPS (L-isomer), DMPS (L-isomer), DOPS (L-isomer), DSPS (D-isomer), DSPG, DPPG, N-Glu-DSPE, and N-Suc-DSPE, optionally wherein the conjugated lipid is PEG-DMG; and the PS lipid is selected from the group consisting of: DSPS (L-isomer) and DPPS,

(ii) wherein the ionizable cationic lipid is one or more compounds selected from the group consisting of: Compounds 1-28 (Table 1), Compounds 29-38 (Table 2), AKG-UO-1,

AKG-UO-1A, AKG-UO-1B, AKG-UO-1B, AKG-UO-2, AKG-UO-3, AKG-UO-4, AKG-UO-4A, AKG-UO-5, AKG-BDG-01, AKG-BDG-02, AKG-UO-6, AKG-UO-7, AKG-UO-8, AKG-UO-9, and AKG-UO-10,

(iii) wherein the ionizable cationic lipid is one or more compounds selected from the group consisting of Compounds 1-3, 5-8, 9-12, 14-28, or

(iv) wherein the ionizable cationic lipid is one or more compounds selected from the group consisting of Compounds 29-38.

7. The composition of any one of claims 1-6, wherein the ionizable cationic lipid is one or more compounds selected from the group consisting of: KC2-OA, KC3-OA, Dlin-KC2-DMA , DlinKC3-DMA, KC2-PA, DODAP, AKG-OA-DM2, AKG-OA-DM3, O-11769, Dlin-MC3-DMA, ALC-0315 and SM-102.
8. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, wherein the LNP composition comprises:
    a. a nucleic acid;
    b. an ionizable cationic lipid in a total amount of 50 mol% of the total lipid content of the LNP composition;
    c. cholesterol in a total amount of 38.5 mol% of the total lipid content of the LNP composition;
    d. one or more phospholipids in a total amount of 7-15 mol% of the total lipid content of the LNP composition, and comprising a phosphatidylserine (PS) lipid in a total amount of 3-9 mol% of the total lipid content of the LNP composition, optionally wherein the phospholipids consist of one or more phospholipids selected from the group consisting of: DSPC, HSPC, egg SM, DPPC and DOPC, optionally wherein the PS lipid is one or more L-serine lipids selected from the group consisting of DPPS and DSPS; and
    e. a PEG-containing lipid in a total amount of 0.5 –1.5 mol% of the total lipid content of the LNP composition; or

wherein the LNP composition comprises one or more phospholipids comprising at least two (L-Serine) PS lipids having mismatched acyl chain lengths, optionally wherein the PS lipids are DPPC and DSPS, and optionally wherein the DPPC and DSPS are each present in the LNP at a total amount of 5 mol% each, based on the total lipid content of the LNP composition.

9. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, comprising:
    a. the nucleic acid, the ionizable cationic lipid AKG-UO-1, and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, optionally wherein the nucleic acid is mRNA, the PS lipid is (L-Serine) DSPS, (L-Serine) DPPS, or a mixture thereof, and the LNP composition further comprises cholesterol and a second phospholipid selected from the group consisting of: DSPC, DPPC and DOPC, and optionally wherein the LNP composition further comprises 0.5-1.5 mol% PEG-DMG or PEG-DSG, based on the total lipid content in the LNP composition; or
    b. a nucleic acid, an ionizable cationic lipid selected from KC2OA, KC2, KC2-01, ALC0315, and SM102; and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, optionally wherein the LNP composition has a N/P ratio of 3 to 8, optionally wherein the LNP composition has a N/P ratio of 5 to 7, optionally wherein the LNP composition has a N/P ratio of 6, optionally wherein the ionizable cationic lipid is KC2OA, KC2, KC2-01, ALC0315, or SM102; or
    c. a nucleic acid, an ionizable cationic lipid selected from AKG-UO-6 and AKG-UO-7; and a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition, optionally wherein the LNP composition has a N/P ratio of 3 to 8, optionally the LNP composition has a N/P ratio of 5 to 7, optionally wherein the LNP composition has a N/P ratio of 5, optionally wherein the LNP composition has a N/P ratio of 7.
10. The composition of any one of claims 1-6 and 8-9, wherein the nucleic acid is a mRNA encoding the SARS-CoV-2 spike protein.
11. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, wherein the composition is a vaccine composition comprising:
    a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
    b. an ALC-0315 ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
    c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;

d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition; or

wherein the LNP vaccine composition comprises:

a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
b. a Dlin-KC3-01 ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

12. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, wherein the composition is a vaccine composition comprising:

a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
b. a KC3-OA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

13. The nucleic acid lipid nanoparticle (LNP) composition of claim 1, wherein the composition is a vaccine composition comprising:
    a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
    b. an ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
    c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
    d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
    e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
    f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition,

    optionally wherein the nucleic acid is a mRNA of SEQ ID NO:2.
14. Use of a (L-Serine) PS lipid in a LNP in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition for targeting of the LNP to dendritic cells.
15. The use of claim 14, wherein the LNP comprises a mRNA, optionally wherein the LNP further comprises cholesterol, optionally wherein the LNP further comprises an ionizable cationic lipid (ICL), optionally wherein the LNP further comprises one or more additional phospholipids including DSPC, optionally wherein the LNP further comprises a conjugated lipid.
16. The use of claim 15, wherein the LNP comprises:
    a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
    b. an ionizable cationic lipid (ICL) in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
    c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
    d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
    e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and

f. a conjugated lipid in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition,

optionally wherein the ICL is selected from the compounds of any one of claims 1-5.

17. A nucleic acid lipid nanoparticle (LNP) vaccine composition comprising an ionizable cationic lipid selected from the group consisting of: KC3-OA, Dlin-KC3-DMA, and KC3-O1, wherein the vaccine composition comprises the ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP vaccine composition.
18. The composition of claim 17, wherein the ionizable cationic lipid is KC3-OA.
19. The composition of claim 17, wherein the ionizable cationic lipid is Dlin-KC3-DMA.
20. The composition of claim 17, wherein the ionizable cationic lipid is KC3-O1.
21. A nucleic acid lipid nanoparticle (LNP) composition comprising a Dlin-KC2-DMA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition, in combination with a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition.
22. The nucleic acid lipid nanoparticle (LNP) vaccine composition of claim 1, wherein the composition is a LNP vaccine composition comprising:
    a. a mRNA nucleic acid with a N/P ratio of 3 to 8;
    b. an ALC-0315 ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
    c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
    d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
    e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
    f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition; or

    wherein the LNP vaccine composition comprises:
    a. a mRNA nucleic acid with a N/P ratio of 3 to 8;

b. a Dlin-KC2-DMA ionizable cationic lipid in a total amount of 40-65 mol% of the total lipid content of the LNP composition;
c. cholesterol in a total amount of 25-40 mol% of the total lipid content of the LNP composition;
d. a (L-Serine) PS lipid in a total amount of 2.5-10 mol% of the total lipid content of the LNP composition;
e. DSPC phospholipid in a total amount of 5-25 mol% of the total lipid content of the LNP composition; and
f. PEG-DMG in a total amount of 0-2.5 mol% of the total lipid content of the LNP composition.

23. A lipid nanoparticle (LNP) composition comprising an ionizable lipid having a chemical structure consisting of a pair of linear polyunsaturated lipid tails covalently bound to a head group, the head group comprising a dialkyl amino group;

the head group comprising a heterocyclyl or alkyl portion covalently bound to the dialkyl amino group and optionally further comprising a phosphate group;

each polyunsaturated lipid tail being unsaturated except for at least two olefins separated by at least two methylene groups along the length of the lipid tail, and optionally comprising a single acyl group at the end of the lipid tail covalently bound to the head group.

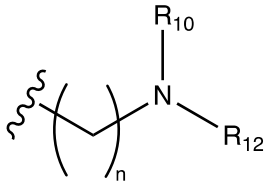
24. The composition of claim 23,

wherein each lipid tail is identical, and each lipid tail has a total of two olefins separated only by an unsubstituted ethylene, n-propyl, or n-butyl,

optionally wherein each lipid tail further comprises an acyl group joined to an oxygen of the headgroup to form an ester, and has a total of 16 or 18 carbon atoms including the acyl group.

25. The composition of claim 24, wherein

a. the dialkyl amino portion of the head group has a chemical structure of Formula (IV-A)

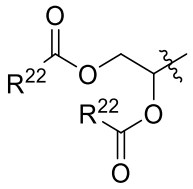


Formula IV-A,

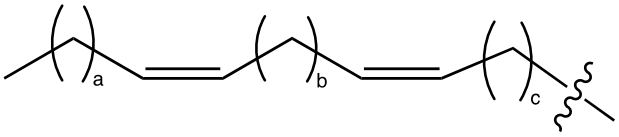
wherein

n is 2, 3 or 4 in Formula (IV-A); and

R_{10} and R_{12} in Formula (IV-A) are each independently selected from an alkyl group selected from the group consisting of: methyl, ethyl, and propyl, wherein the alkyl in R_{10} and R_{12} is optionally substituted with one or more hydroxyl; and

b. the ionizable lipid further comprises the chemical structure 

comprising the acyl group of each lipid tail covalently bound to the portion of the head group distal to the dialkyl amino portion of Formula (IV-A), wherein  indicates attachment to Formula IV-A within the head group, and R^{22} indicates a portion of each lipid tail covalently bound to the acyl group and having the chemical structure of Formula A:



Formula A

wherein  in Formula A indicates attachment of Formula A to R^{22} within each lipid tail; and

a is 4, 1, 2, or 3;

b is 4, 2, or 3; and

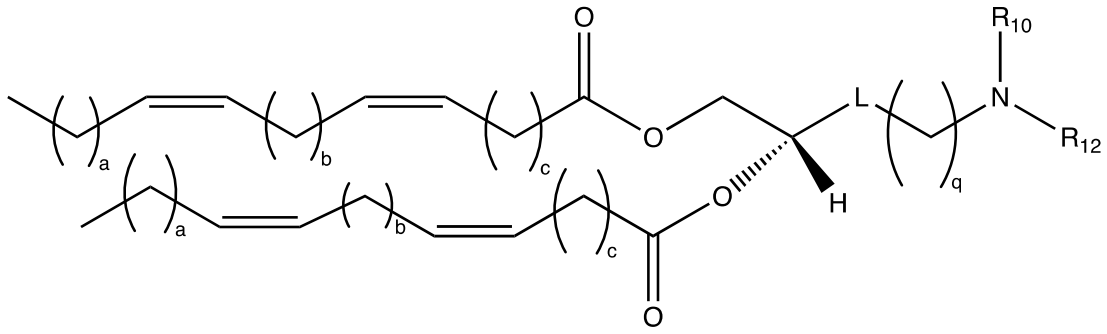
c is 4, 3, 5, 6, or 7,

provided that the sum of a, b and c is in Formula A is 12, 10, 11, or 13,

optionally wherein R_{10} and R_{12} in Formula (IV-A) are each independently methyl, ethyl, -$(CH_2)(CH_2)$OH, or –$(CH_2)_2(CH_2)$OH, and

optionally wherein b is 4 and wherein R_{10} and R_{12} in Formula (IV-A) are each methyl.

26. The composition of claim 23, wherein the ionizable lipid has a chemical structure of Formula (I-A)



Formula I-A

wherein

a is 4, 1, 2, 3, 5 or 6;

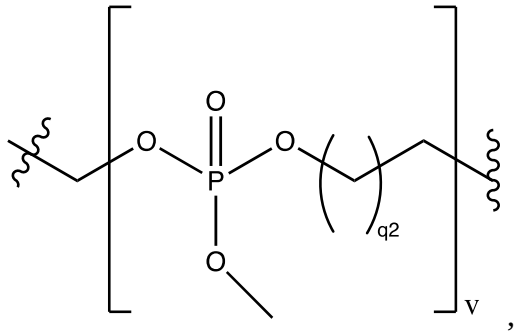
b is 4, 3 or 2;

c is 4, 3, 5, 6, or 7; and

the sum of a, b and c is 12 or 10;

q is 1, 2, 3 or 4; and

each of R_{10} and R_{12} is independently (C_1-C_4)alkyl optionally substituted with one or more hydroxyl; and

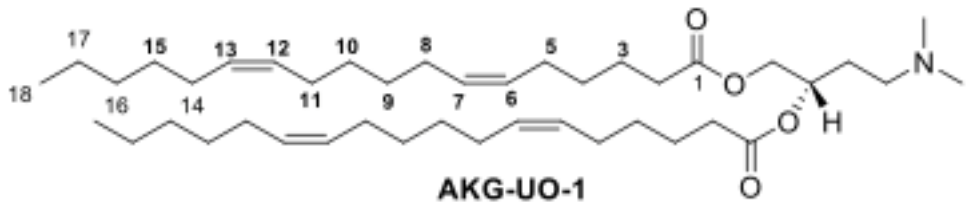
L is 

,

wherein v is 0 or 1; and q2 is 2 or 1,

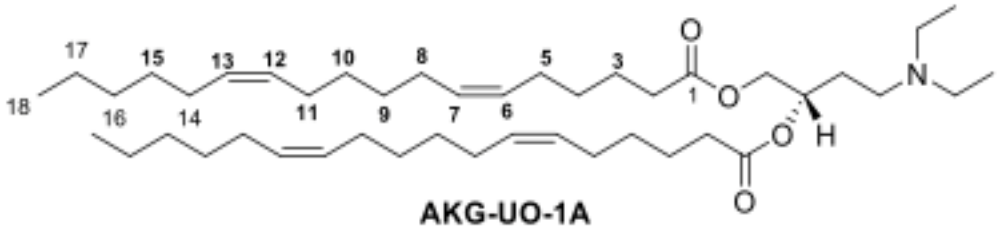
optionally wherein v is 0 and q is 1, 2 or 3.

27. The composition of claim 26, wherein the ionizable lipid is selected from the group consisting of AKG-UO-1, AKG-UO-1A, AKG-UO-1B, AKG-UO-2, AKG-UO-4, AKG-UO-4A, AKG-UO-5, AKG-UO-6, AKG-UO-7, AKG-UO-8, AKG-UO-9, and AKG-UO-10:



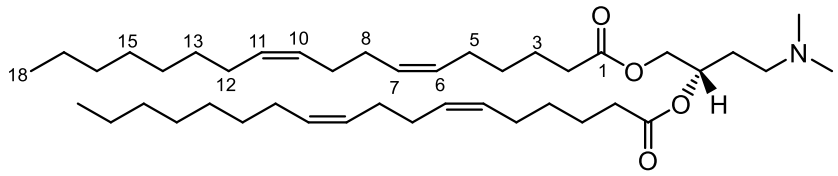
AKG-UO-1

;

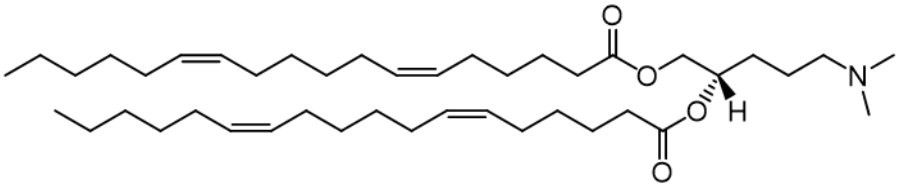


AKG-UO-1A

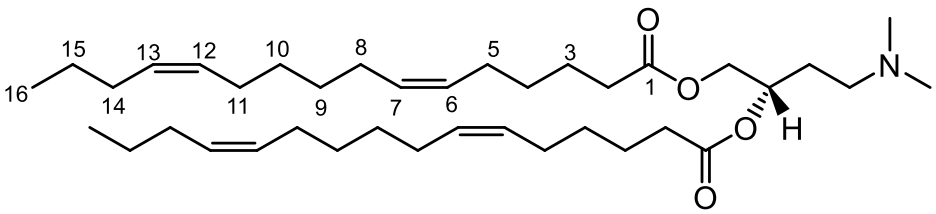
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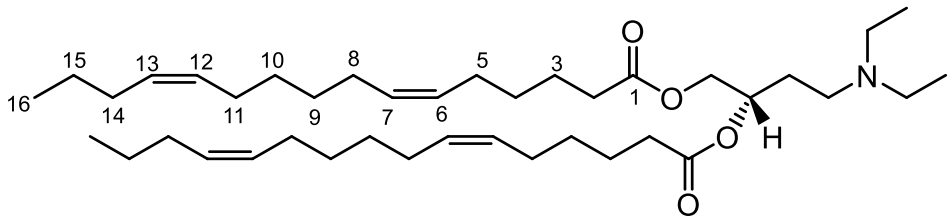
AKG-UO-1B;



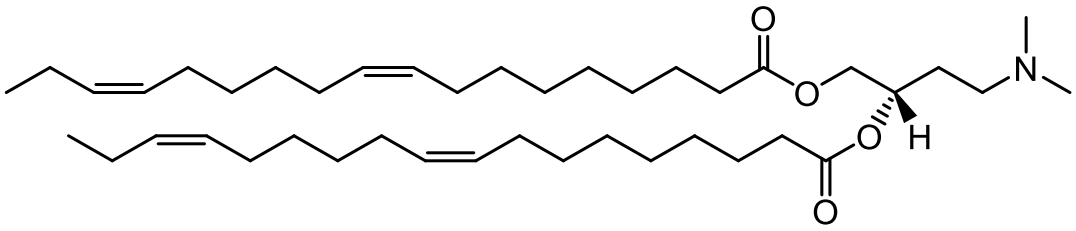
AKG-UO-2;



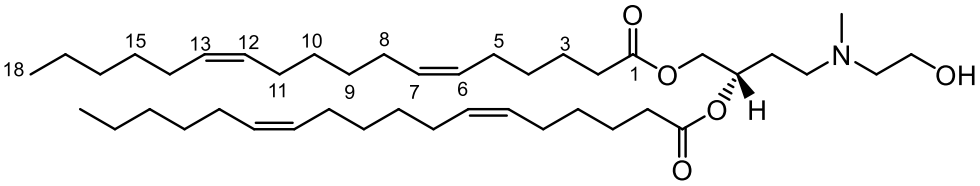
AKG-UO-4;



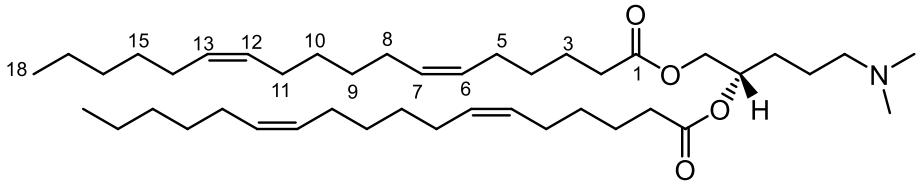
AKG-UO-4A;



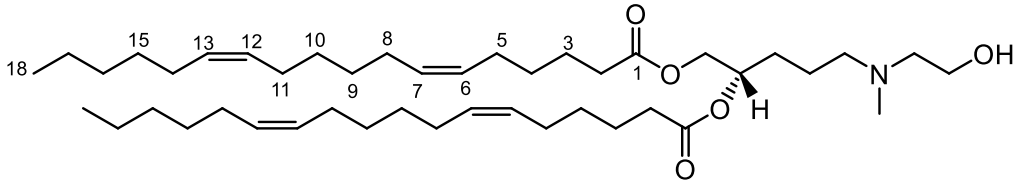
AKG-UO-5;



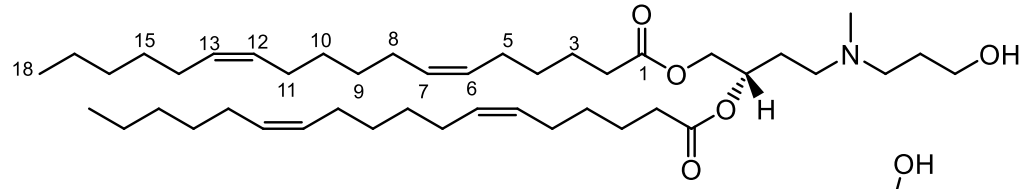
AKG-UO-6;



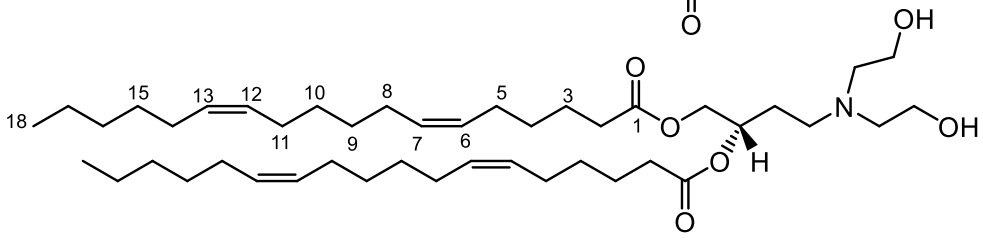
AKG-UO-7;



AKG-UO-8;



AKG-UO-9;



AKG-UO-10.

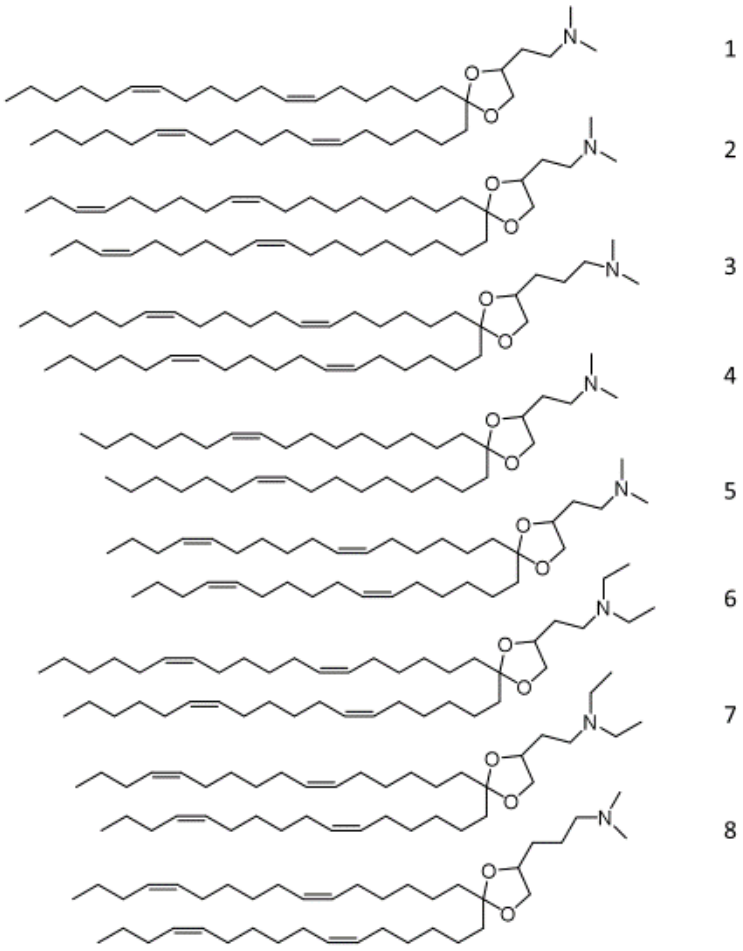
28. The composition of any one of claims 23-27, comprising:

a. a nucleic acid;

b. the ionizable lipid of any one of claims 23-27;
c. a sterol, optionally wherein the sterol is cholesterol;
d. one or more phospholipids comprising a phosphatidylserine (PS) lipid, optionally wherein the one or more phospholipids consist of (i) one or more phospholipids selected from the group consisting of: DSPC, HSPC, DPPC, egg SM, and DOPC, and (ii) a PS lipid selected from the group consisting of: DPPS, DSPS and DOPS; and
e. optionally further comprising a conjugated lipid.

29. The composition of claim 28, wherein the one or more phospholipids consist of:
a. DSPC; and
b. one or more PS lipids selected from the group consisting of (L-Serine) DPPS and (L-Serine) DSPS,
wherein optionally the composition comprises the PS lipid in a total amount of 2.5-10 mol% of the total lipid in the composition,
optionally wherein the conjugated lipid comprises PEG.

30. The composition of claim 23, wherein the ionizable lipid is selected from the group consisting of:



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31. The composition of claim 28, wherein the one or more phospholipids consist of:

a. HSPC; and

b. one or more PS lipids selected from the group consisting of (L-Serine) DPPS and (L-Serine) DSPS,

wherein optionally the composition comprises the PS lipid in a total amount of 2.5-10 mol% of the total lipid in the composition,

optionally wherein the conjugated lipid comprises PEG.