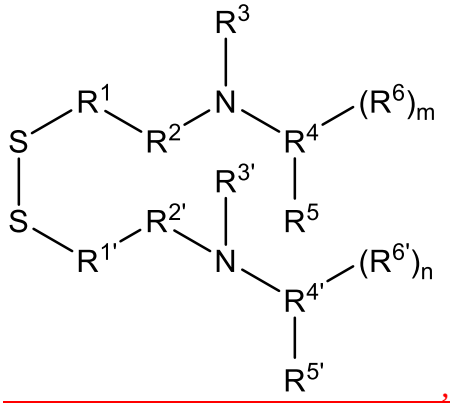
with reduced toxicity, but with improved pharmacokinetics and intracellular kinetics such as cell uptake and nucleic acid release from the lipid carrier.

SUMMARY

In one aspect of the present disclosure, there is provided an ionizable lipid represented by Formula (I):



,

or a pharmaceutically acceptable salt thereof, wherein:

R^1 and $R^{1'}$ are each independently linear or branched C_{1-3} alkylene;

R^2 and $R^{2'}$ are each independently linear or branched C_{1-6} alkylene;

R^3 and $R^{3'}$ are each independently linear or branched C_{1-6} alkyl;

or alternatively, when R^2 is branched C_{1-6} alkylene, R^2 and R^3, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{2'}$ is branched C_{1-6} alkylene, $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R^4 and $R^{4'}$ are each independently $–CR^a$, $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$;

R^a, for each occurrence, is independently H or C_{1-3} alkyl;

or alternatively, when R^4 is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, R^3 and R^4, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

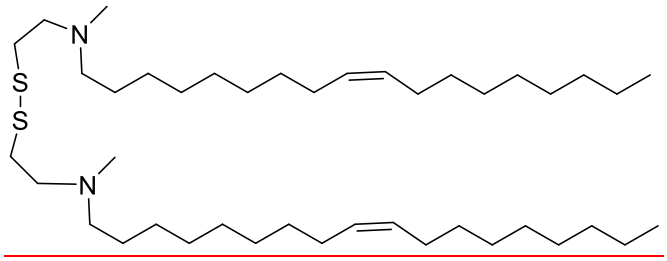
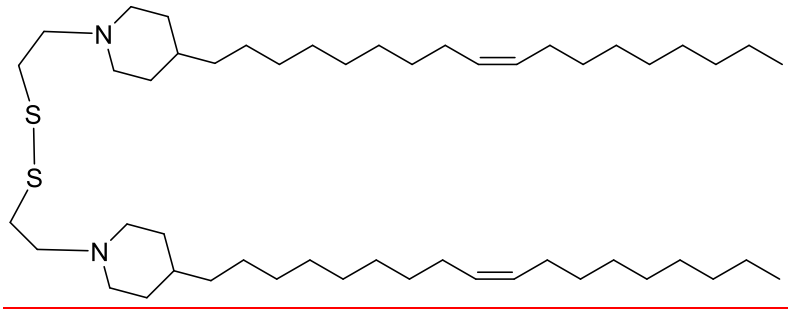
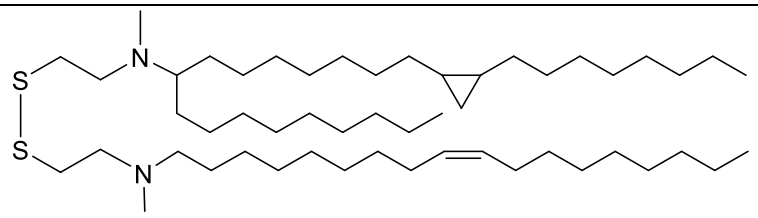
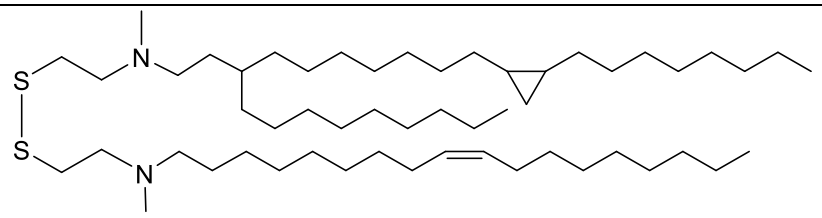
or alternatively, when $R^{4'}$ is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

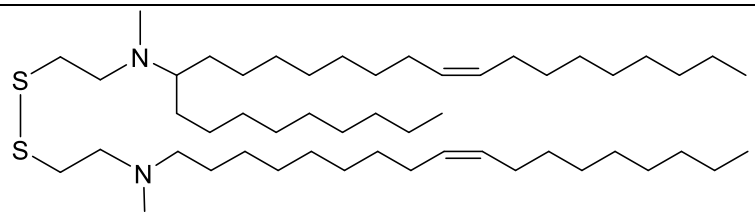
R^5 and $R^{5'}$ are each independently C_{4-20} alkylene or C_{4-20} alkenylene;

R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20} alkenylene; and

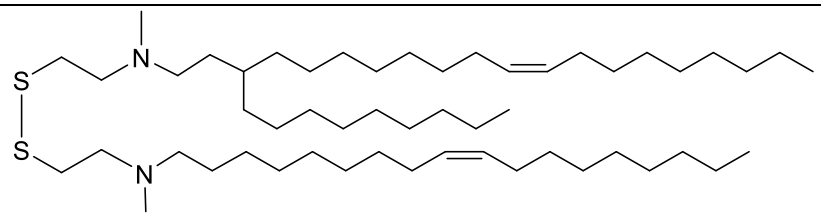
m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

In another aspect of the present disclosure, there is provided an ionizable lipid selected from:

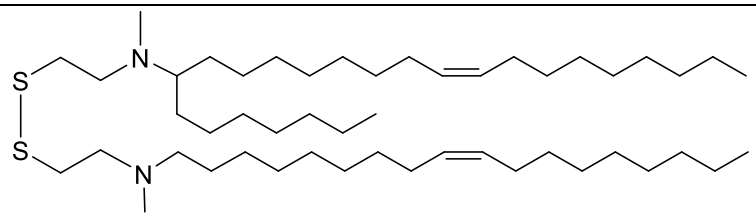
 (9Z,9'Z)-N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyloctadec-9-en-1-amine);
 1,2-bis(2-(4-((Z)-octadec-9-en-1-yl)piperidin-1-yl)ethyl)disulfane;
 (Z)-N-methyl-N-(2-((2-(methyl(1-(2-octylcyclopropyl)heptadecan-8-yl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 (Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;



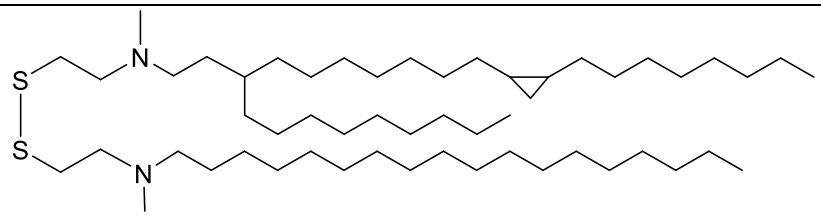
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)heptacos-18-en-10-amine;



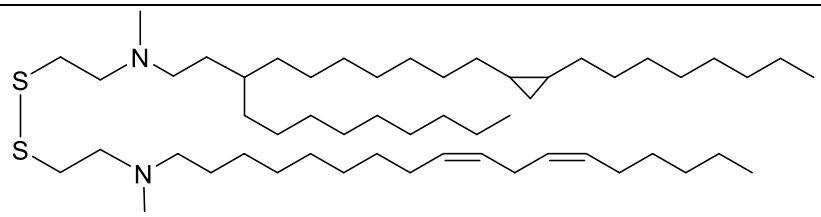
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)-3-nonylicos-11-en-1-amine;



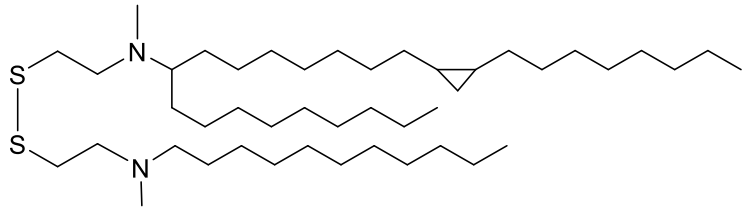
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)pentacos-16-en-8-amine;



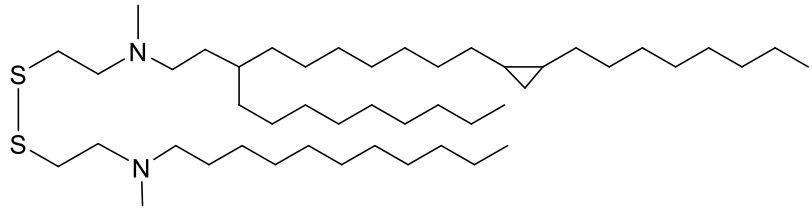
N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;



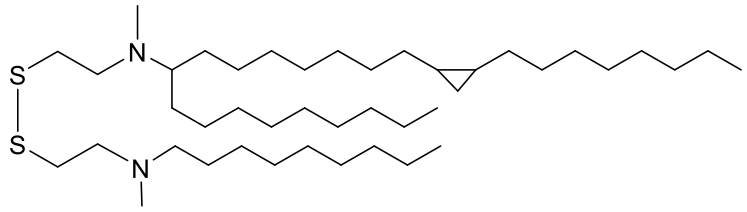
(9Z,12Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine;



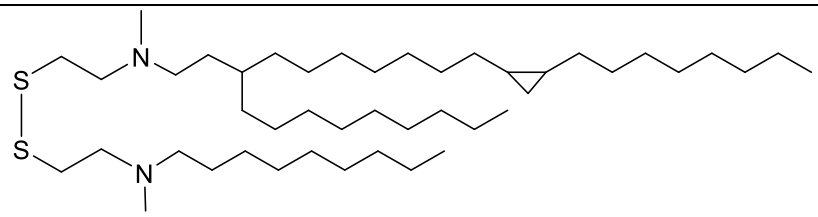
N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;



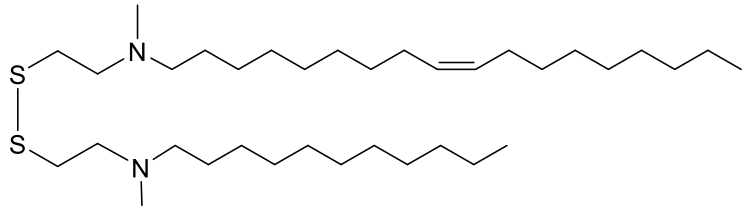
N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;



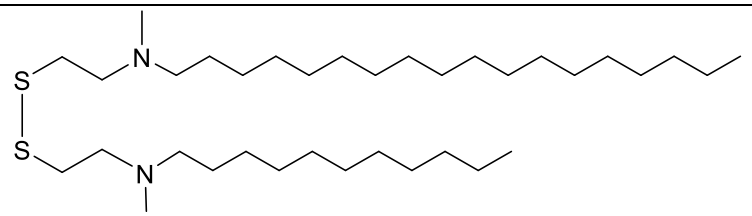
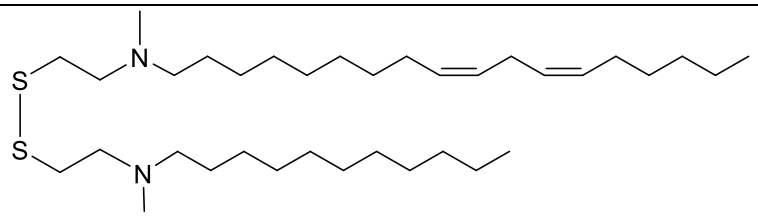
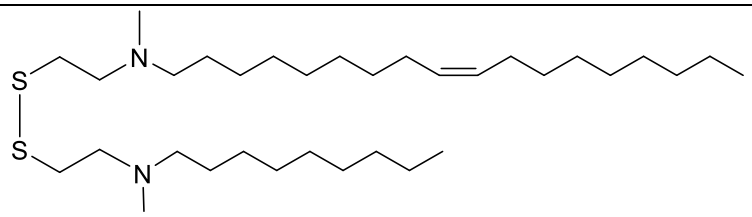
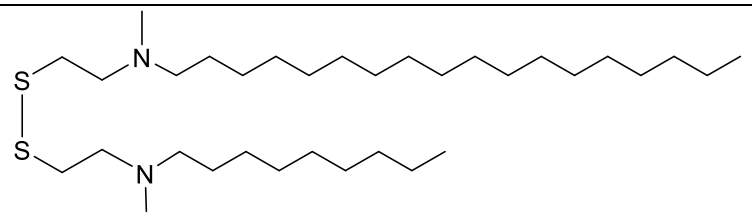
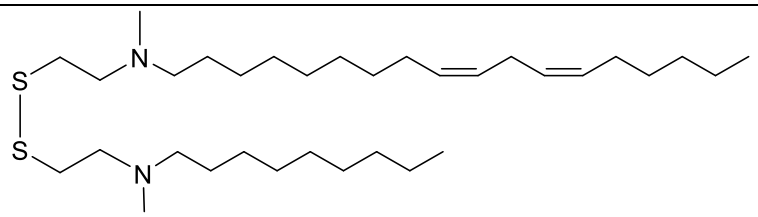
N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;



N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;



(Z)-N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;

 N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;
 (9Z,12Z)-N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine;
 (Z)-N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine; and
 (9Z,12Z)-N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine.

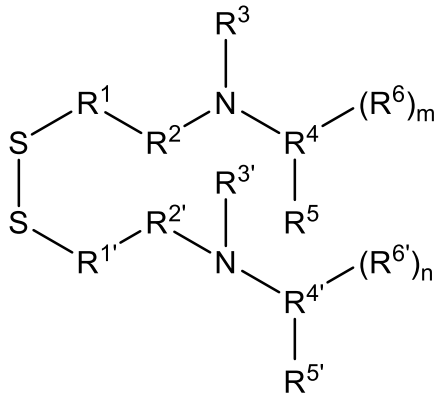
In a further aspect of the present disclosure, there is provided a lipid nanoparticle (LNP) comprising the ionizable lipid disclosed herein and a nucleic acid.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the lipid nanoparticle disclosed herein and a pharmaceutically acceptable excipient.

In a further aspect of the present disclosure, there is provided a use of a lipid nanoparticle disclosed herein or an effective amount of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a genetic disorder in a subject.

In another aspect of the present disclosure, there is provided a lipid nanoparticle disclosed herein, or an effective amount of the pharmaceutical composition disclosed herein, for use in therapy.

Provided herein is an ionizable lipid represented by Formula (I):



,

or a pharmaceutically acceptable salt thereof, wherein:

R^1 and $R^{1'}$ are each independently optionally substituted linear or branched C_{1-3} alkylene;

R^2 and $R^{2'}$ are each independently optionally substituted linear or branched C_{1-6} alkylene;

R^3 and $R^{3'}$ are each independently optionally substituted linear or branched C_{1-6} alkyl;

or alternatively, when R^2 is optionally substituted branched C_{1-6} alkylene, R^2 and R^3, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{2'}$ is optionally substituted branched C_{1-6} alkylene, $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R^4 and $R^{4'}$ are each independently $–CR^a$, $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$;

R^a, for each occurrence, is independently H or C_{1-3} alkyl;

or alternatively, when R^4 is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, R^3 and R^4, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{4'}$ is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R^5 and $R^{5'}$ are each independently C_{1-20} alkylene or C_{2-20} alkenylene;

R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20} alkenylene; and

m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^2 and $R^{2'}$ are each independently C_{1-3} alkylene.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the linear or branched C_{1-3} alkylene represented by R^1 or $R^{1'}$, the linear or branched C_{1-6} alkylene represented by R^2 or $R^{2'}$, and the optionally substituted linear or branched C_{1-6} alkyl are each optionally substituted with one or more halo and cyano groups.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^1 and R^2 taken together are C_{1-3} alkylene and $R^{1'}$ and $R^{2'}$ taken together are C_{1-3} alkylene, *e.g.*, ethylene.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^3 and $R^{3'}$ are each independently optionally substituted C_{1-3} alkyl, *e.g.*, methyl.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^4 and $R^{4'}$ are each –CH.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^2 is optionally substituted branched C_{1-6} alkylene; and R^2 and R^3, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, $R^{2'}$ is optionally substituted branched C_{1-6} alkylene; and $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl, such as pyrrolidinyl or piperidinyl.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^4 is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and R^a is C_{1-3} alkyl; and R^3 and R^4, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, $R^{4'}$ is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and R^a is C_{1-3} alkyl; and $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl, such as pyrrolidinyl or piperidinyl.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^5 and $R^{5'}$ are each independently C_{1-10} alkylene or C_{2-10} alkenylene. In one ~~embodiment~~example, R^5 and $R^{5'}$ are each independently C_{1-8} alkylene or C_{1-6} alkylene.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, R^6 and $R^{6'}$, for each occurrence, are independently C_{1-10} alkylene, C_{3-10} cycloalkylene, or C_{2-10} alkenylene. In one ~~embodiment~~example, C_{1-6} alkylene, C_{3-6} cycloalkylene, or C_{2-6} alkenylene. In one ~~embodiment~~example the C_{3-10} cycloalkylene or the C_{3-6} cycloalkylene is cyclopropylene. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, m and n are each 3.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the ionizable lipid is selected from any one of the lipids in Table 1 or a pharmaceutically acceptable salt thereof.

Another ~~aspect~~example of the present disclosure relates to a lipid nanoparticle (LNP) comprising an ionizable lipid of Formula (I) including any of the ~~aspect~~examples or ~~embodiment~~examples herein and a nucleic acid. In one ~~embodiment~~example, the nucleic acid is encapsulated in the ionizable lipid. In a particular ~~embodiment~~example, the nucleic acid is a closed-ended DNA (ceDNA).

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP further comprises a sterol. According to some ~~embodiment~~examples, the sterol can be a cholesterol, or beta-sitosterol.

According to some ~~embodiment~~examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 35%, about 25% to about 30%, or about 30% to about 35%, and the ionizable lipid is present at a molar percentage of about 80% to about 60%, for example about 80% to about 65%, about 80% to about 70%, about 80% to about 75%, about 75% to about 60%, about 75% to about 65%, about 75% to about 70%, about 70% to about 60%, or about 70% to about 60%. According to some ~~embodiment~~examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%, and wherein the ionizable lipid is present at a molar percentage of about 80% to about 60%, for example about 80%, about 79%, about 78%, about 77%, about 76%, about 75%, about 74%, about 73%, about 72%, about 71%, about 70%, about 69%, about 68%, about 67%, about 66%, about 65%, about 64%, about 63%, about 62%, about 61%, or about 60%. According to some ~~embodiment~~examples, the cholesterol is present at a molar percentage of about 40%, and wherein the ionizable lipid is present at a molar percentage of about 50%.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the composition further comprises a cholesterol, a PEG or PEG-lipid conjugate, and a non-cationic lipid. According to some ~~embodiment~~examples, the PEG or PEG-lipid conjugate is present at about 1.5% to about 3%, for example about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about

2.75%, or about 2.25% to about 2.5%. According to some ~~embodiment~~examples, the PEG or PEG-lipid conjugate is present at about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3%. According to some ~~embodiment~~examples, the cholesterol is present at a molar percentage of about 30% to about 50%, for example about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 20% to about 40%, about 40% to about 50%, or about 45% to about 50%. According to some ~~embodiment~~examples, the cholesterol is present at a molar percentage of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, or about 50%.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP further comprises a polyethylene glycol (PEG). According to some ~~embodiment~~examples, the PEG is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG or DMG-PEG2000).

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP further comprises a non-cationic lipid. According to some ~~embodiment~~examples, the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3 -pho sphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-

phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof. According to some ~~embodiment~~examples, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

According to some ~~embodiment~~examples, the PEG or PEG-lipid conjugate is present at about 1.5% to about 4%, for example about 1.5% to about 3%, about 2% to about 3%, about 2.5% to about 3%, about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 1.5% to about 1.75%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%. According to some ~~embodiment~~examples, the PEG or PEG-lipid conjugate is present at about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3%. According to some ~~embodiment~~examples, the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%. According to some ~~embodiment~~examples, the ionizable lipid is present at a molar percentage of about 42.5%, about 43%, about 43.5%, about 44%, about 44.5%, about 45%, about 45.5%, about 46%, about 46.5%, about 47%, about 47.5%, about 48%, about 48.5%, about 49%, about 49.5%, about 50%, about 50.5%, about 51%, 51.5%, about 52%, about 52.5%, about 53%, about 53.5%, about 54%, about 54.5%, about 55%, about 55.5%, about 56%, about 56.5%, about 57%, 57.5%, about 58%, about 58.5%, about 59%, about 59.5%, about 60%, about 60.5%, about 61%, about 61.5%, about 62%, or about 62.5%. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the cholesterol is present at a molar percentage of about 40%, the ionizable lipid is present at a molar percentage of about 52.5%, the non-cationic lipid is present at a molar percentage of about 7.5%, and wherein the PEG is present at about 3%.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition further comprises dexamethasone palmitate.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP is in size ranging from about 50 nm to about 110 nm in diameter, for example about 50 nm to about 100 nm, about 50 nm to about 95 nm, about 50 nm to about 90 nm, about 50 nm

to about 85 nm, about 50 nm to about 80 nm, about 50 nm to about 75 nm, about 50 nm to about 70 nm, about 50 nm to about 65 nm, about 50 nm to about 60 nm, about 50 nm to about 55 nm, about 60 nm to about 110 nm, about 60 nm to about 100 nm, about 60 nm to about 95 nm, about 60 nm to about 90 nm, about 60 nm to about 85 nm, about 60 nm to about 80 nm, about 60 nm to about 75 nm, about 60 nm to about 70 nm, about 60 nm to about 65 nm, about 70 nm to about 110 nm, about 70 nm to about 100 nm, about 70 nm to about 95 nm, about 70 nm to about 90 nm, about 70 nm to about 85 nm, about 70 nm to about 80 nm, about 70 nm to about 75 nm, about 80 nm to about 110 nm, about 80 nm to about 100 nm, about 80 nm to about 95 nm, about 80 nm to about 90 nm, about 80 nm to about 85 nm, about 90 nm to about 110 nm, or about 90 nm to about 100 nm. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP is less than about 100 nm in size, for example less than about 105 nm, less than about 100 nm, less than about 95 nm, less than about 90 nm, less than about 85 nm, less than about 80 nm, less than about 75 nm, less than about 70 nm, less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some ~~embodiment~~examples, the LNP is less than about 70 nm in size., for example less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some ~~embodiment~~examples, the LNP is less than about 60 nm in size, for example less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 10:1. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 20:1. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 30:1. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 40:1. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the composition has a total lipid to nucleic acid ratio of about 50:1.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP further comprises a tissue targeting moiety. The tissue targeting moiety can be a peptide,

oligosaccharide or the like, which can be used for the delivery of the LNP to one or more specific tissues such as cancer, the liver, the CNS, or the muscle. According to some ~~embodiment~~examples, the tissue targeting moiety is a ligand for liver specific receptors. According to one ~~embodiment~~example, the ligand of liver specific receptors used for liver targeting is an oligosaccharide such as N-Acetylgalactosamine (GalNAc) which is covalently attached to a component of a LNP, e.g., PEG-lipid conjugates or the like. According to some ~~embodiment~~examples, the GalNAc is covalently attached to, for example, PEG-lipid conjugate. Accordingly to some ~~embodiment~~examples, the GalNAc is conjugated to DSPE-PEG2000. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the lipid nanoparticle at a molar percentage of 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.2% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.3% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.4% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.5% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.6% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.7% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.8% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 0.9% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 1.0% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of about 1.5% of the total lipid. According to some ~~embodiment~~examples, the GalNAc-PEG-lipid conjugate is present in the LNP at a molar percentage of 2.0% of the total lipid.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition is prepared in a buffer such as malic acid. In some ~~embodiment~~examples, the composition is prepared in about 10 mM to about 30 mM malic acid, for example about 10 mM to about 25 mM, about 10 mM to about 20 mM, about 10 mM to about 15 mM, about 15 mM to about 25 mM, about 15 mM to about 20 mM, about 20 mM to about 25 mM. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the

composition is prepared in about 10 mM malic acid, about 11 mM malic acid, about 12 mM malic acid, about 13 mM malic acid, about 14 mM malic acid, about 15 mM malic acid, about 16 mM malic acid, about 17 mM malic acid, about 18 mM malic acid, about 19 mM malic acid, about 20 mM malic acid, about 21 mM malic acid, about 22 mM malic acid, about 23 mM malic acid, about 24 mM malic acid, about 25 mM malic acid, about 26 mM malic acid, about 27 mM malic acid, about 28 mM malic acid, about 29 mM malic acid, or about 30 mM malic acid. According to some ~~embodiment~~examples, the composition comprises about 20 mM malic acid.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having about 30 mM to about 50 mM NaCl, for example about 30 mM to about 45 mM NaCl, about 30 mM to about 40 mM NaCl, about 30 mM to about 35 mM NaCl, about 35 mM to about 45 mM NaCl, about 35 mM to about 40 mM NaCl, or about 40 mM to about 45 mM NaCl. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the LNP composition is prepared in a solution having about 30 mM NaCl, about 35 mM NaCl, about 40 mM NaCl, or about 45 mM NaCl. According to some ~~embodiment~~examples, the LNP composition is prepared in a solution having about 40 mM NaCl.

According to some ~~embodiment~~examples, the LNP composition is prepared in a solution having about 20 mM to about 100 mM $MgCl_2$, for example about 20 mM to about 90 mM $MgCl_2$, about 20 mM to about 80 mM $MgCl_2$, about 20 mM to about 70 mM $MgCl_2$, about 20 mM to about 60 mM $MgCl_2$, about 20 mM to about 50 mM $MgCl_2$, about 20 mM to about 40 mM $MgCl_2$, about 20 mM to about 30 mM $MgCl_2$, about 320 mM to about 90 mM $MgCl_2$, about 30 mM to about 80 mM $MgCl_2$, about 30 mM to about 70 mM $MgCl_2$, about 30 mM to about 60 mM $MgCl_2$, about 30 mM to about 50 mM $MgCl_2$, about 30 mM to about 40 mM $MgCl_2$, about 40 mM to about 90 mM $MgCl_2$, about 40 mM to about 80 mM $MgCl_2$, about 40 mM to about 70 mM $MgCl_2$, about 40 mM to about 60 mM $MgCl_2$, about 40 mM to about 50 mM $MgCl_2$, about 50 mM to about 90 mM $MgCl_2$, about 50 mM to about 80 mM $MgCl_2$, about 50 mM to about 70 mM $MgCl_2$, about 50 mM to about 60 mM $MgCl_2$, about 60 mM to about 90 mM $MgCl_2$, about 60 mM to about 80 mM $MgCl_2$, about 60 mM to about 70 mM $MgCl_2$, about 70 mM to about 90 mM $MgCl_2$, about 70 mM to about 80 mM $MgCl_2$, or about 80 mM to about 90 mM $MgCl_2$.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the ceDNA is closed-ended linear duplex DNA. According to some ~~embodiment~~examples of any of the

~~aspect~~examples or ~~embodiment~~examples herein, the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.

According to some ~~embodiment~~examples, the ceDNA comprises expression cassette comprising a polyadenylation sequence.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette. According to some ~~embodiment~~examples, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. According to some ~~embodiment~~examples, the expression cassette is connected to an ITR at 3' end (3' ITR). According to some ~~embodiment~~examples, the expression cassette is connected to an ITR at 5' end (5' ITR). According to some ~~embodiment~~examples, at least one of 5' ITR and 3' ITR is a wild-type AAV ITR. According to some ~~embodiment~~examples, at least one of 5' ITR and 3' ITR is a modified ITR. According to some ~~embodiment~~examples, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

According to some ~~embodiment~~examples, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. According to some ~~embodiment~~examples, the spacer sequence is at least 5 base pairs long in length. According to some ~~embodiment~~examples, the spacer sequence is 5 to 100 base pairs long in length. According to some ~~embodiment~~examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 base pairs long in length. According to some ~~embodiment~~examples, the spacer sequence is 5 to 500 base pairs long in length. According to some ~~embodiment~~examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, or 495 base pairs long in length.

According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the ceDNA has a nick or a gap.

According to some ~~embodiment~~examples, the ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, a wild-type ITR from a parvovirus. According to some ~~embodiment~~examples, the AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.

According to some ~~embodiment~~examples, the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR. According to some ~~embodiment~~examples, the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette, optionally wherein the two mutant ITRs are symmetric mutants. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministering DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA. According to some ~~embodiment~~examples of any of the ~~aspect~~examples or ~~embodiment~~examples herein, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

According to some ~~aspect~~examples, the ~~disclosure~~present disclosure provides a method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the pharmaceutical composition according to any of the ~~aspect~~examples or ~~embodiment~~examples herein. According to some ~~embodiment~~examples, the subject is a human. According to some ~~embodiment~~examples, the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, mucopolysaccharide storage diseases (*e.g.*, Hurler syndrome (MPS Type I), Scheie syndrome (MPS Type I S), Hurler-Scheie syndrome (MPS Type I H-S), Hunter syndrome (MPS Type II), Sanfilippo Types A, B, C, and D (MPS Types III A, B, C, and D), Morquio Types A and B (MPS IVA and MPS IVB), Maroteaux-Lamy syndrome (MPS Type VI), Sly syndrome (MPS Type VII), hyaluronidase deficiency (MPS Type IX)), Niemann-Pick Disease Types A/B, C1 and C2, Fabry disease, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease Types I and II (Pompe disease), Gaucher disease Types I, II and III, Fabry disease, cystinosis, Batten disease, Aspartylglucosaminuria, Salla disease, Danon disease (LAMP-2 deficiency), Lysosomal Acid Lipase (LAL) deficiency, neuronal ceroid lipofuscinoses (CLN1-8, INCL, and LINCL), sphingolipidoses, galactosialidosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, spinocerebellar ataxia, spinal muscular atrophy,

Friedreich's ataxia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophies (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2) and Cathepsin A deficiency. According to some ~~embodiment~~examples, the genetic disorder is Leber congenital amaurosis (LCA). According to some ~~embodiment~~examples, the LCA is LCA10. According to some ~~embodiment~~examples, the genetic disorder is Niemann-Pick disease. According to some ~~embodiment~~examples, the genetic disorder is Stargardt macular dystrophy. According to some ~~embodiment~~examples, the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II). According to some ~~embodiment~~examples, the genetic disorder is hemophilia A (Factor VIII deficiency). According to some ~~embodiment~~examples, the genetic disorder is hemophilia B (Factor IX deficiency). According to some ~~embodiment~~examples, the genetic disorder is hunter syndrome (Mucopolysaccharidosis II). According to some ~~embodiment~~examples, the genetic disorder is cystic fibrosis. According to some ~~embodiment~~examples, the genetic disorder is dystrophic epidermolysis bullosa (DEB). According to some ~~embodiment~~examples, the genetic disorder is phenylketonuria (PKU). According to some ~~embodiment~~examples, the genetic disorder is progressive familial intrahepatic cholestasis (PFIC). According to some ~~embodiment~~examples, the genetic disorder is Wilson disease. According to some ~~embodiment~~examples, the genetic disorder is Gaucher disease Type I, II or III.

BRIEF DESCRIPTION OF THE DRAWINGS

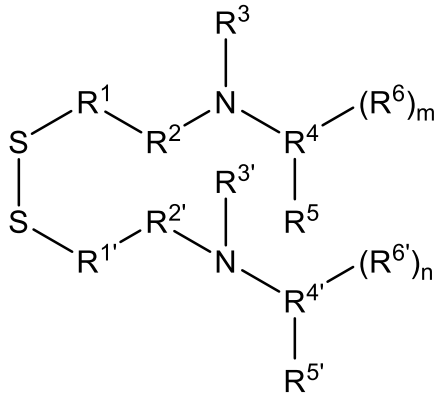
Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

FIG. 1A illustrates an exemplary structure of a ceDNA vector for expression of a transgene as disclosed herein, comprising asymmetric ITRs. In this embodiment, the exemplary ceDNA vector comprises an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene can be inserted into the cloning site (R3/R4) between the CAG promoter and WPRE. The expression cassette

CLAIMS

What is Claimed is:

1. An ionizable lipid represented by Formula (I):



,

or a pharmaceutically acceptable salt thereof, wherein:

R^1 and $R^{1'}$ are each independently ~~optionally substituted~~ linear or branched C_{1-3} alkylene;

R^2 and $R^{2'}$ are each independently ~~optionally substituted~~ linear or branched C_{1-6} alkylene;

R^3 and $R^{3'}$ are each independently ~~optionally substituted~~ linear or branched C_{1-6} alkyl;

or alternatively, when R^2 is ~~optionally substituted~~ branched C_{1-6} alkylene, R^2 and R^3, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{2'}$ is ~~optionally substituted~~ branched C_{1-6} alkylene, $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R^4 and $R^{4'}$ are each independently $–CR^a$, $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$;

R^a, for each occurrence, is independently H or C_{1-3} alkyl;

or alternatively, when R^4 is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, R^3 and R^4, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{4'}$ is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R^5 and $R^{5'}$ are each independently C_{4-20}~~C_{1-20}~~ alkylene or C_{4-20}~~C_{2-20}~~ alkenylene;

R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20} alkenylene; and

m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

~~2. The ionizable lipid according to claim 1, wherein the linear or branched C_{1-3} alkylene represented by R^1 or $R^{1'}$, the linear or branched C_{1-6} alkylene represented by R^2 or $R^{2'}$, and the optionally substituted linear or branched C_{1-6} alkyl are each optionally substituted with one or more halo and cyano groups.~~

~~3~~2. The ionizable lipid according to claim 1, wherein R^2 and $R^{2'}$ are each independently C_{1-3} alkylene; or R^1 and R^2 taken together are C_{1-3} alkylene and $R^{1'}$ and $R^{2'}$ taken together are C_{1-3} alkylene.

~~4. The ionizable lipid according to claim 3, wherein R^1 and R^2 taken together are C_{1-3} alkylene and $R^{1'}$ and $R^{2'}$ taken together are C_{1-3} alkylene.~~

~~5. The ionizable lipid according to claim 4, wherein R^1 and R^2 taken together are ethylene and $R^{1'}$ and $R^{2'}$ taken together are ethylene.~~

~~6. The ionizable lipid according to claim 1, wherein R^3 and $R^{3'}$ are each independently optionally substituted C_{1-3} alkyl.~~

~~7~~3. The ionizable lipid according to claim ~~6~~2, wherein R^3 and $R^{3'}$ are each methyl.

~~8~~4. The ionizable lipid according to claim 1, wherein R^4 and $R^{4'}$ are each –CH.

~~9~~5. The ionizable lipid according to claim 1, wherein R^2 is optionally substituted branched C_{1-6} alkylene; and wherein R^2 and R^3, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

~~10~~6. The ionizable lipid according to claim 1, wherein $R^{2'}$ is optionally substituted branched C_{1-6} alkylene; and wherein $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

~~11~~7. The ionizable lipid according to claim 1, wherein R^4 is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and R^a is C_{1-3} alkyl; and wherein R^3 and R^4, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

~~12~~8. The ionizable lipid according to claim 1, wherein $R^{4'}$ is $–C(R^a)_2CR^a$, or $–[C(R^a)_2]_2CR^a$ and R^a is C_{1-3} alkyl; and wherein $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

~~13~~9. The ionizable lipid according to any one of claims ~~9-12~~5-8, wherein the 5- or 6-membered heterocyclyl is pyrrolidinyl or piperidinyl.

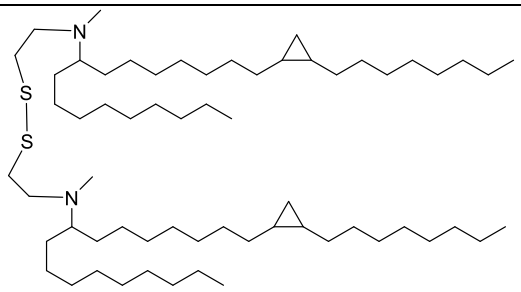
~~14~~10. The ionizable lipid according to any one of claims ~~1-13~~1-9, wherein R^5 and $R^{5'}$ are each independently C_{4-9}~~C_{1-10}~~ alkylene or C_{4-9}~~C_{2-10}~~ alkenylene.

~~15~~11. The ionizable lipid according to any one of claims ~~1-14~~1-10, wherein R^6 and $R^{6'}$, for each occurrence, are independently C_{1-10} alkylene, C_{3-10} cycloalkylene, or C_{2-10} alkenylene.

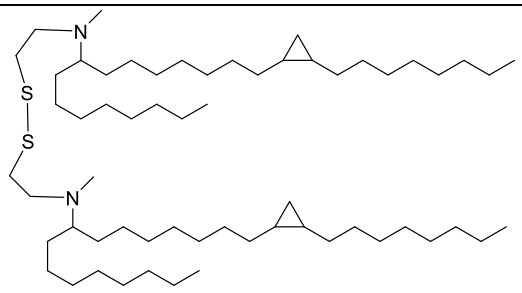
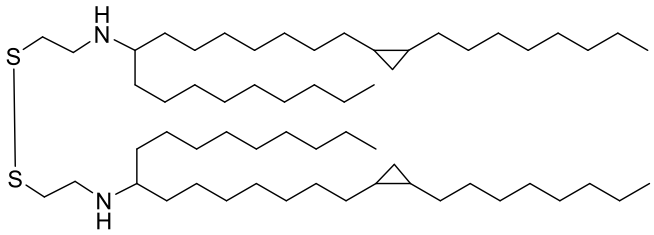
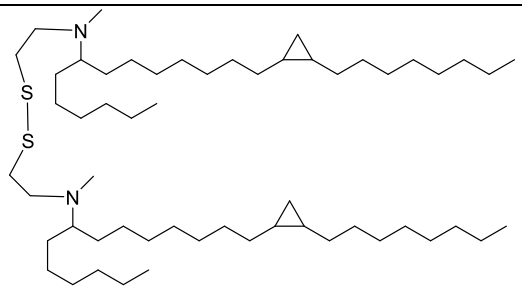
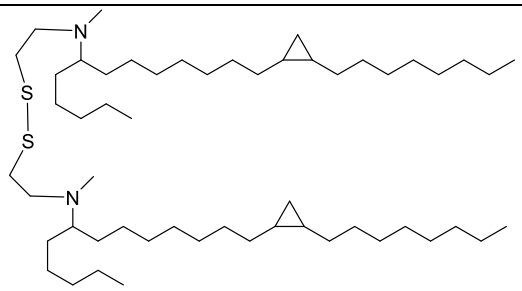
~~16~~12. The ionizable lipid according to claim ~~15~~11, wherein the C_{3-10} cycloalkylene is cyclopropylene.

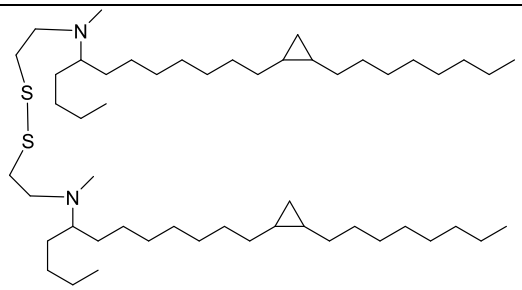
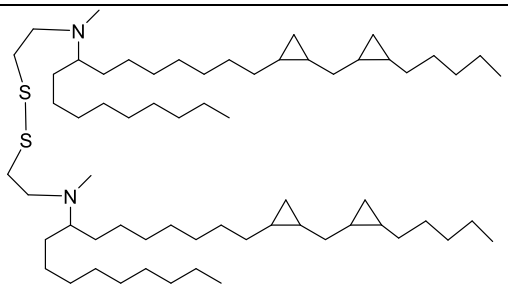
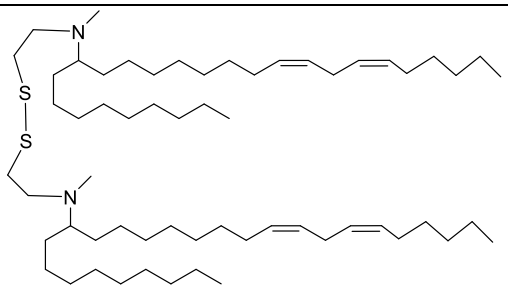
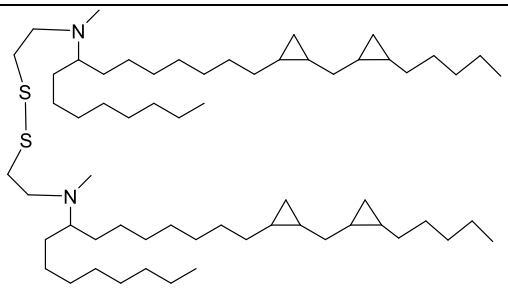
~~17~~13. The ionizable lipid according to claim ~~15~~11 or claim ~~16~~12, wherein m and n are each 3 or 5.

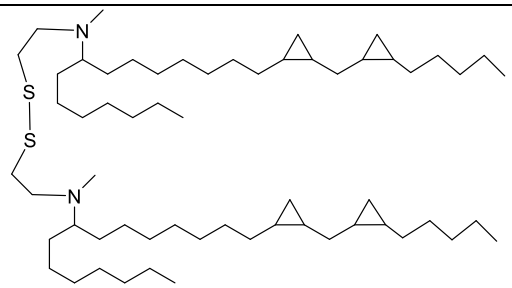
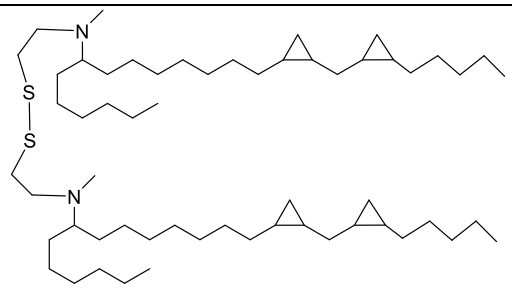
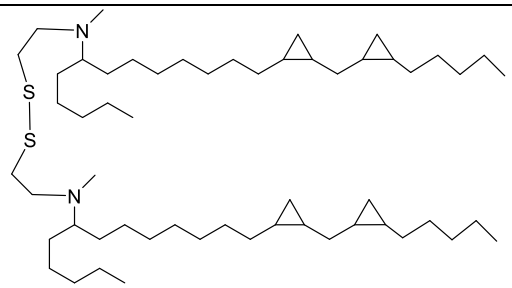
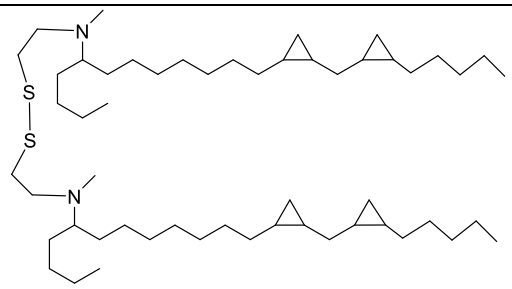
~~18~~14. The ionizable lipid according to claim 1~~Claim 1~~, wherein the ionizable lipid is selected from:

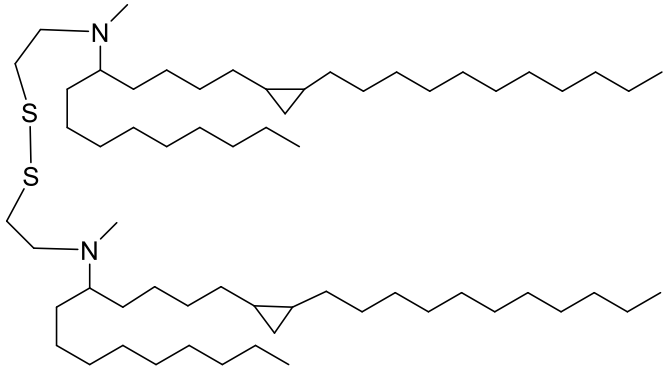
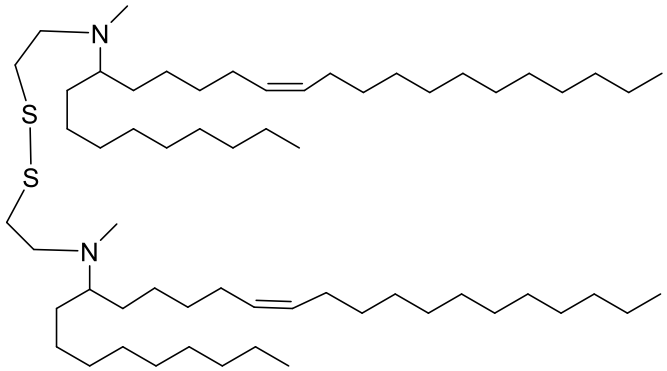
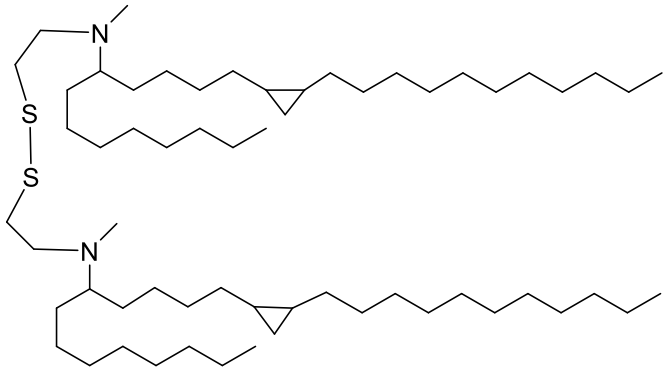


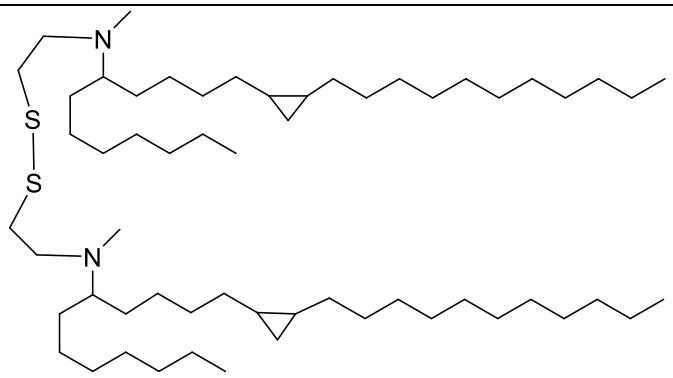
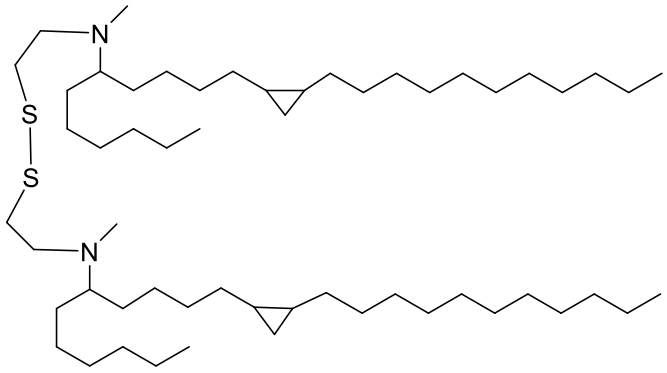
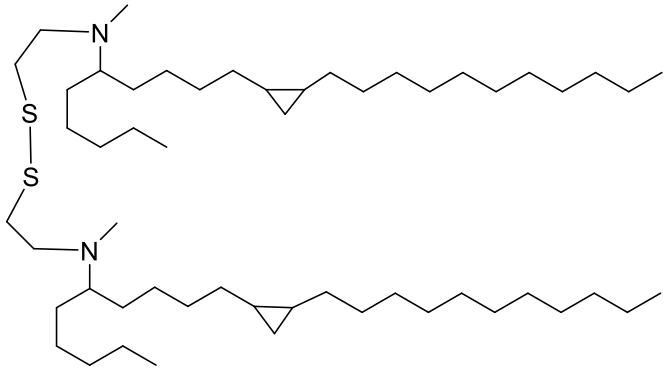
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-8-amine);

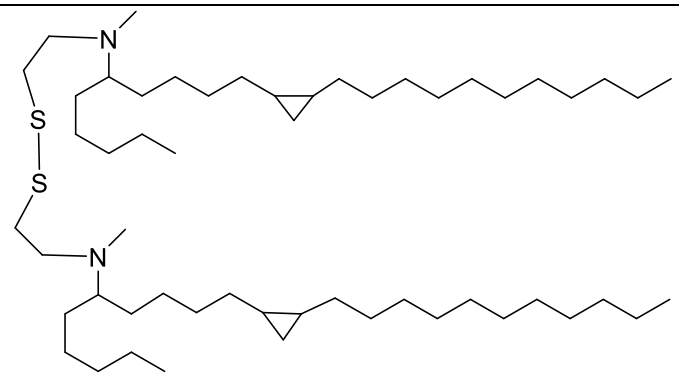
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)hexadecan-8-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(1-(2-octylcyclopropyl)heptadecan-8-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-14-(2-octylcyclopropyl)tetradecan-7-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-13-(2-octylcyclopropyl)tridecan-6-amine);

 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-12-(2-octylcyclopropyl)dodecan-5-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)heptadecan-8-amine);
 (18Z,18'Z,21Z,21'Z)-N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methylheptacosa-18,21-dien-10-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)hexadecan-8-amine);

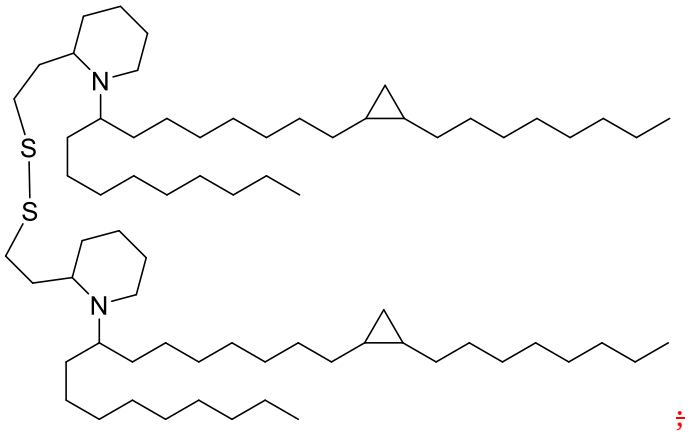
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)pentadecan-8-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-14-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)tetradecan-7-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-13-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)tridecan-6-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-12-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)dodecan-5-amine);

 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)tetradecan-5-amine);
 (15Z,15'Z)-N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methylheptacos-15-en-10-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)tridecan-5-amine);

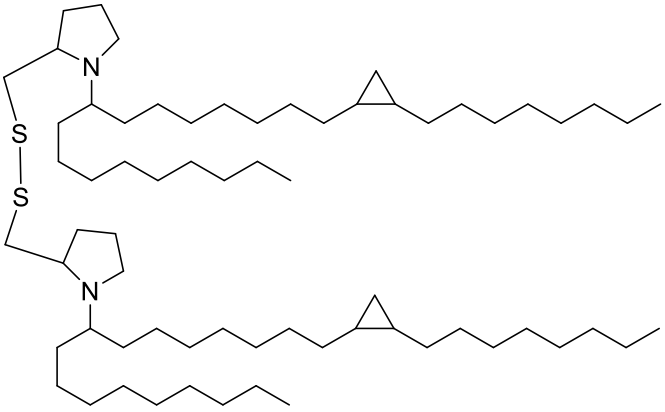
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)dodecan-5-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)undecan-5-amine);
 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)decan-5-amine);



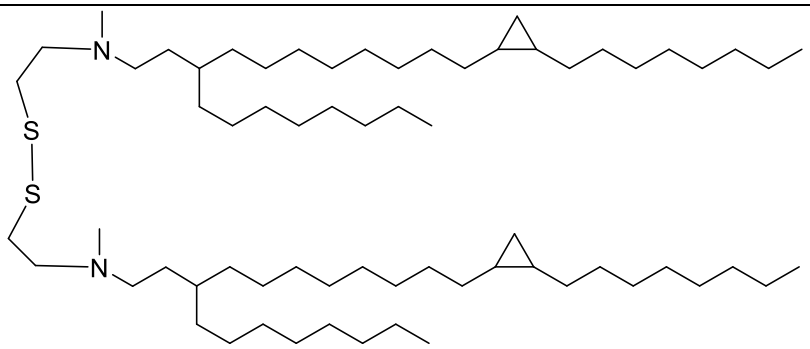
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)decan-5-amine);



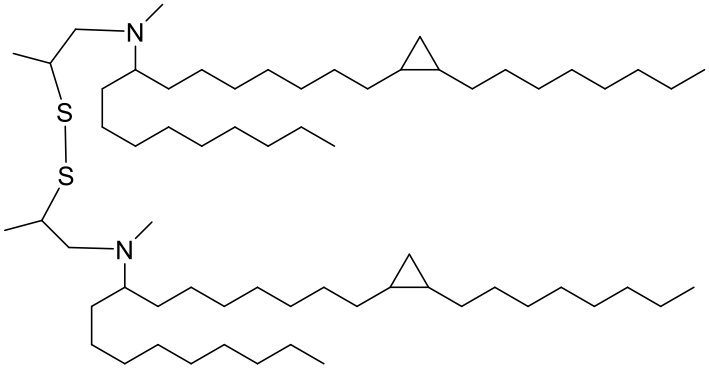
1,2-bis(2-(1-(1-(2-octylcyclopropyl)heptadecan-8-yl)piperidin-2-yl)ethyl)disulfane;



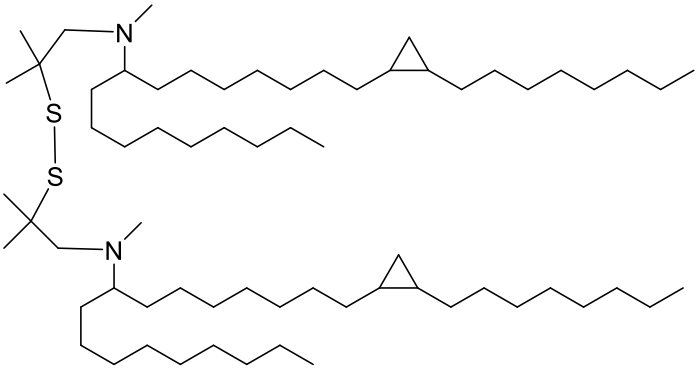
1,2-bis((1-(1-(2-octylcyclopropyl)heptadecan-8-yl)pyrrolidin-2-yl)methyl)disulfane;



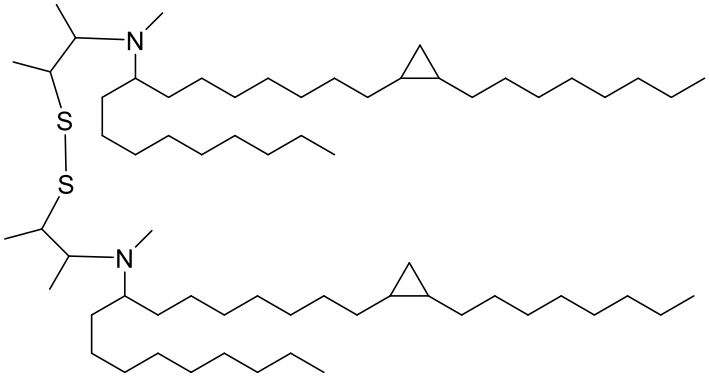
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-3-octyl-11-(2-octylcyclopropyl)undecan-1-amine);



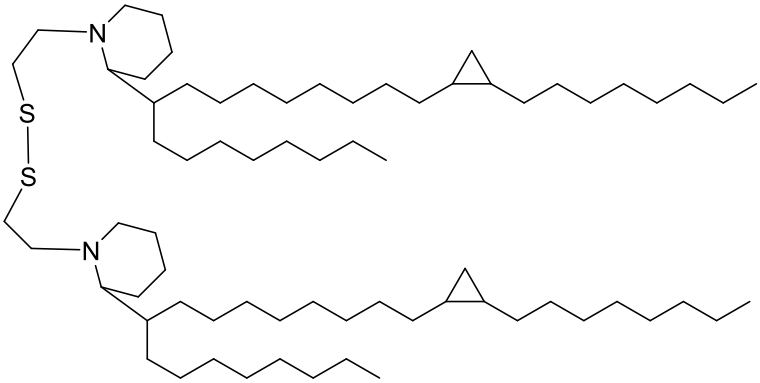
N,N'-(disulfanediylbis(propane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-9-amine);



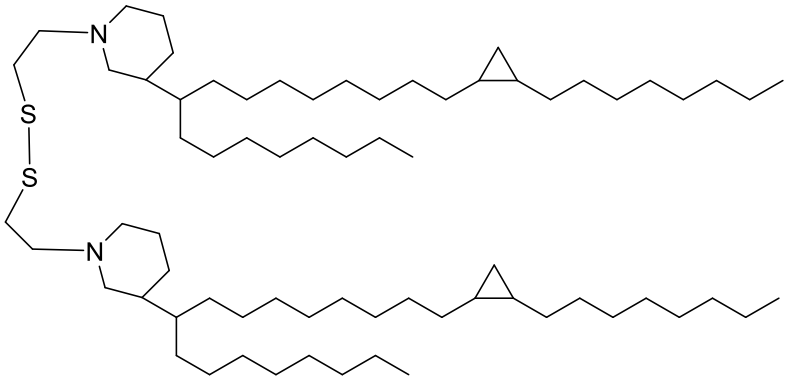
N,N'-(disulfanediylbis(2-methylpropane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-8-amine);



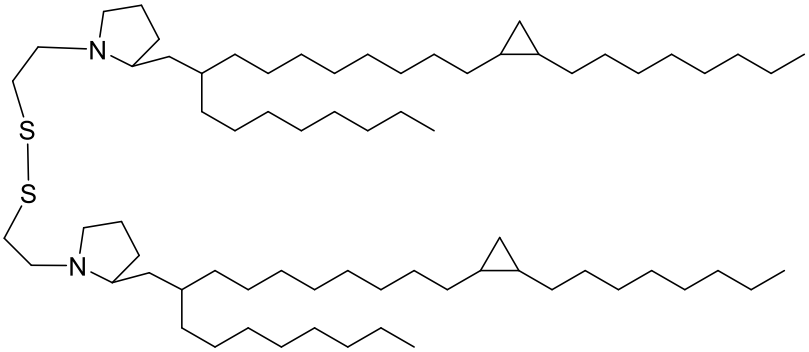
N,N'-(disulfanediylbis(butane-3,2-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-8-amine);



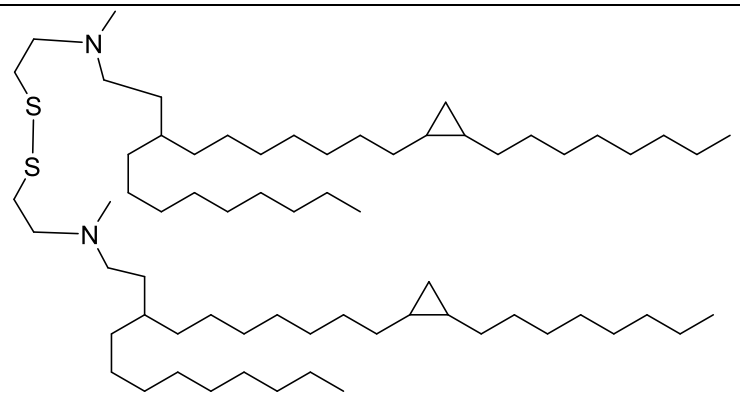
1,2-bis(2-(2-(1-(2-octylcyclopropyl)heptadecan-9-yl)piperidin-1-yl)ethyl)disulfane;



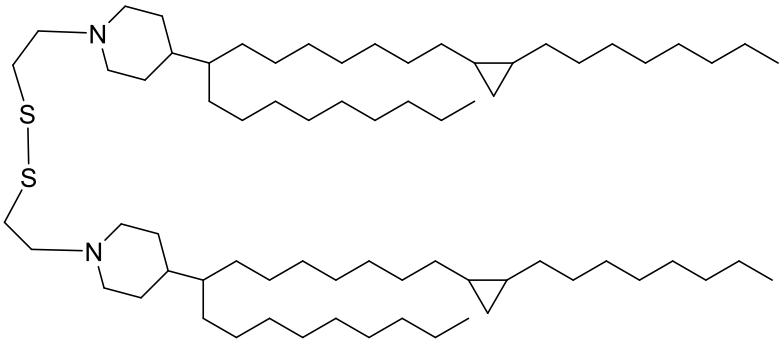
1,2-bis(2-(3-(1-(2-octylcyclopropyl)heptadecan-9-yl)piperidin-1-yl)ethyl)disulfane; ~~and~~



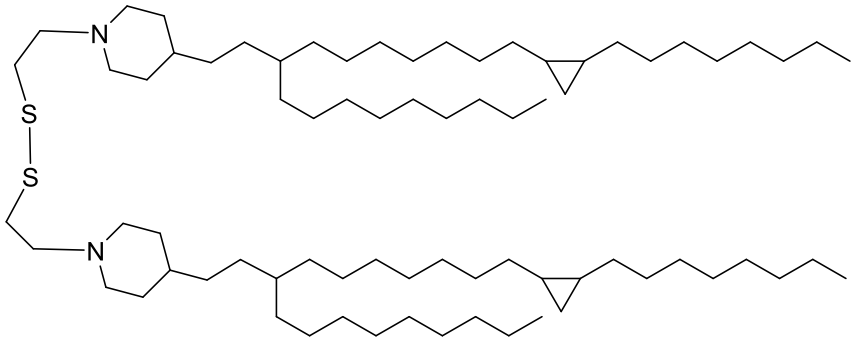
1,2-bis(2-(2-(2-octyl-10-(2-octylcyclopropyl)decyl)pyrrolidin-1-yl)ethyl)disulfane;



N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine);



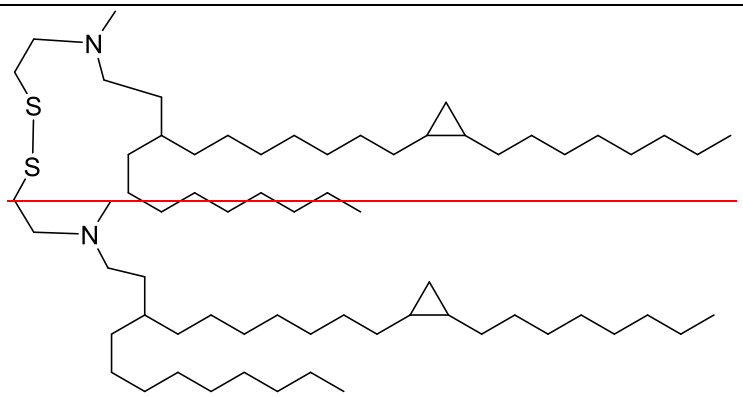
1,2-bis(2-(4-(1-(2-octylcyclopropyl)heptadecan-8-yl)piperidin-1-yl)ethyl)disulfane; and



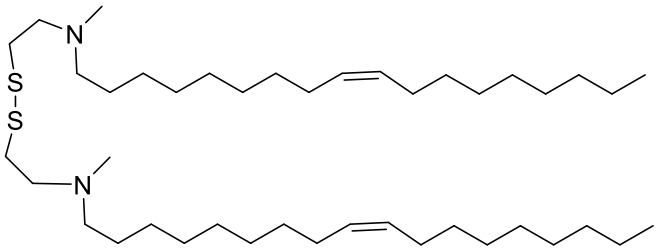
1,2-bis(2-(4-(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)piperidin-1-yl)ethyl)disulfane;

or a pharmaceutically acceptable salt of any of the foregoing.

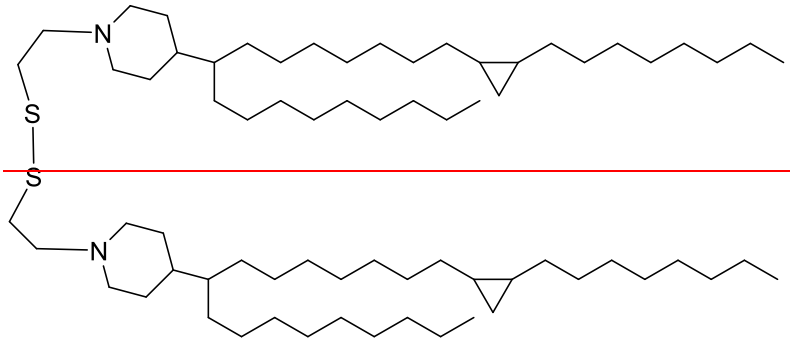
~~19~~15. ~~The ionizable lipid according to Claim 1, wherein the~~An ionizable lipid is selected from:



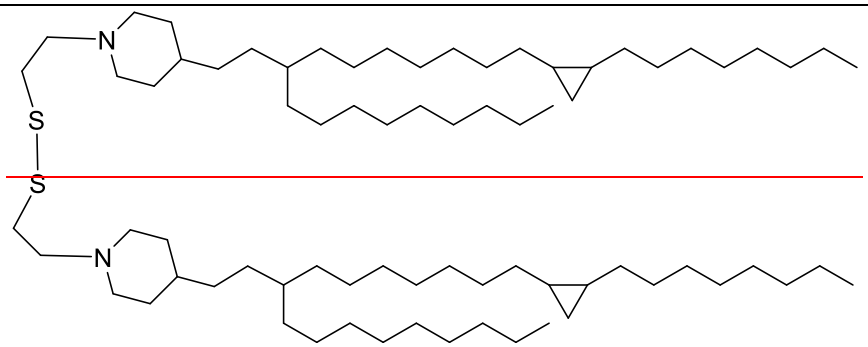
~~N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine);~~



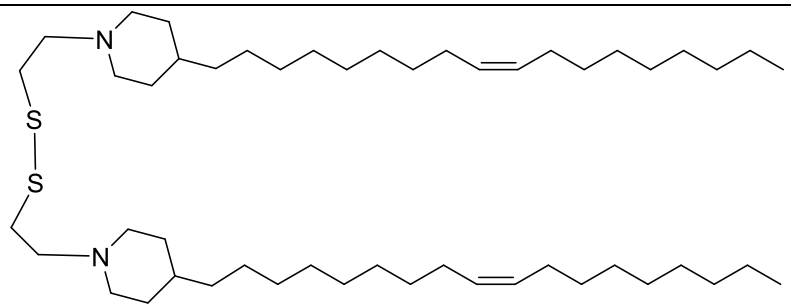
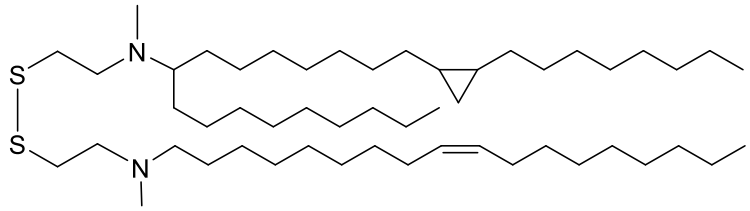
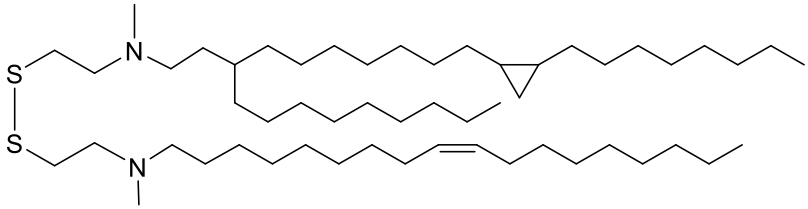
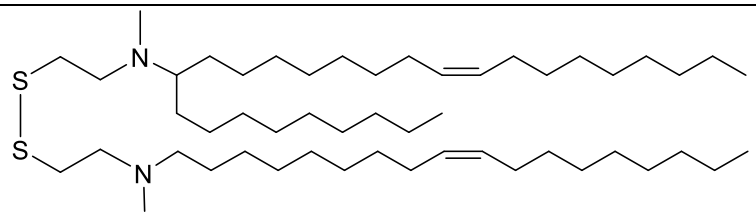
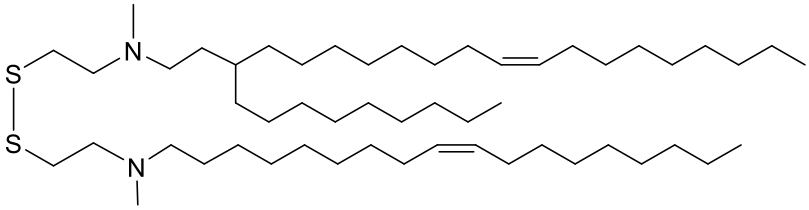
(9Z,9'Z)-N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyloctadec-9-en-1-amine);

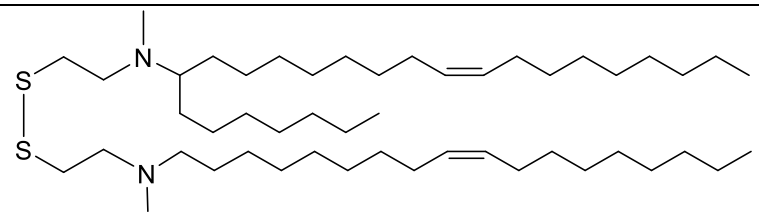
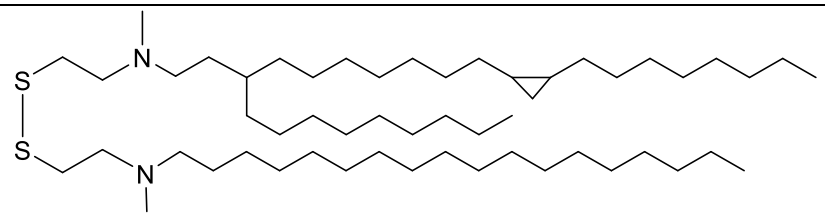
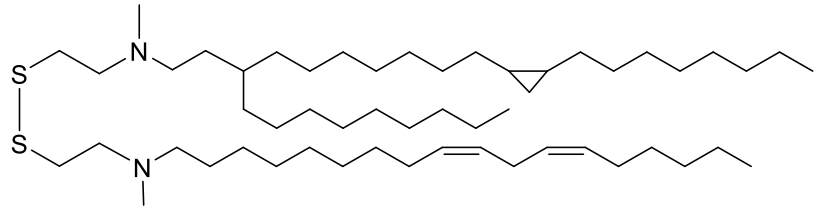
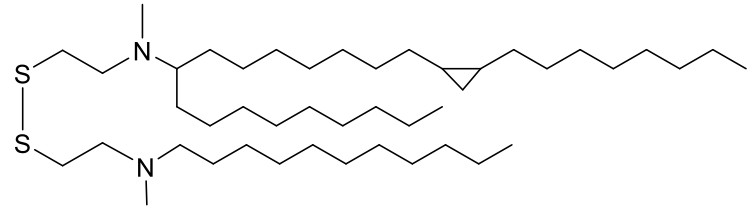
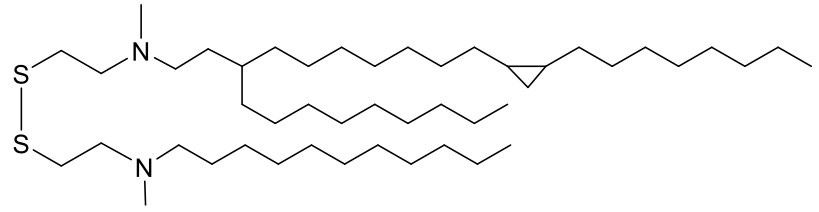


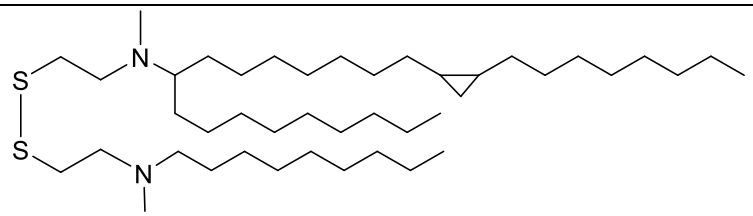
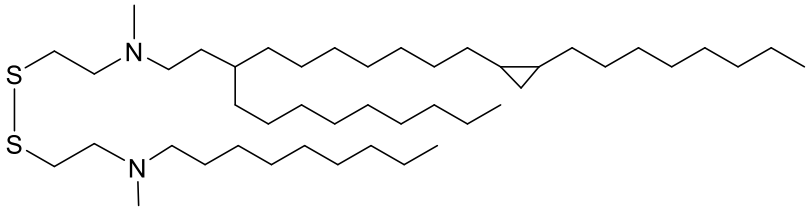
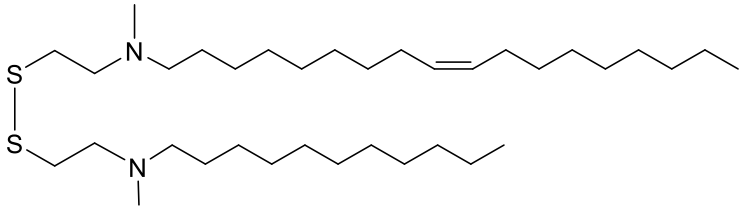
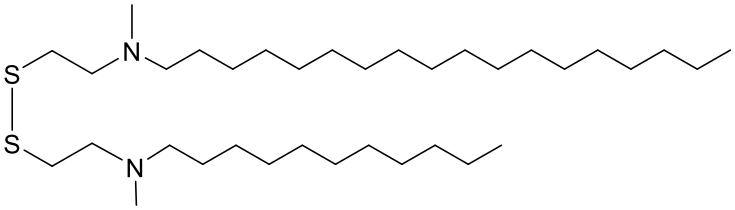
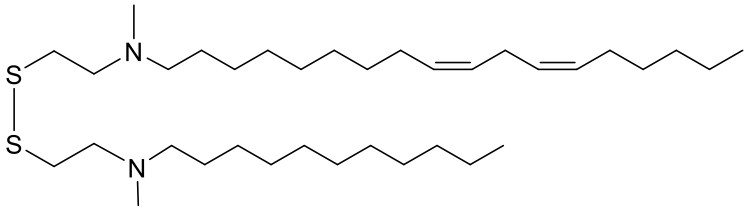
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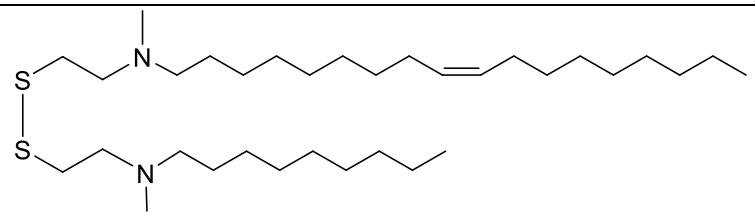
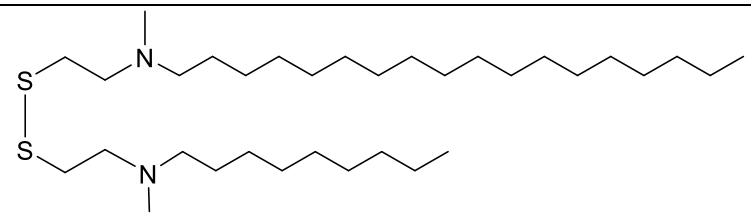
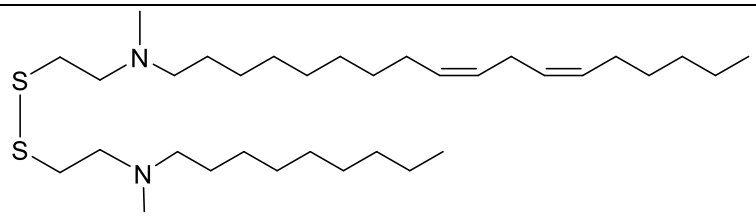


~~1,2-bis(2-(4-(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)piperidin-1-yl)ethyl)disulfane;~~

 1,2-bis(2-(4-((Z)-octadec-9-en-1-yl)piperidin-1-yl)ethyl)disulfane;
 (Z)-N-methyl-N-(2-((2-(methyl(1-(2-octylcyclopropyl)heptadecan-8-yl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 (Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 (Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)heptacos-18-en-10-amine;
 (Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)-3-nonylicos-11-en-1-amine;

 (Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)pentacos-16-en-8-amine;
 N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;
 (9Z,12Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine;
 N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;
 N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;

 N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;
 N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;
 (Z)-N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;
 (9Z,12Z)-N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine;

 (Z)-N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;
 N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine; and
 (9Z,12Z)-N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine.

~~20~~16. A lipid nanoparticle (LNP) comprising the ionizable lipid of any one of claims ~~1-19~~1-15 ~~and 101~~ and a nucleic acid.

~~21~~17. The lipid nanoparticle according to claim ~~20~~16, wherein the nucleic acid is encapsulated in the ionizable lipid.

~~22~~18. The lipid nanoparticle according to claim ~~20~~16 or claim ~~21~~17, wherein the nucleic acid is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, ceDNA, ministring, doggybone™, protelomere closed ended DNA, or dumbbell linear DNA, dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering

RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, DNA viral vectors, viral RNA vector, non-viral vector and any combination thereof.

~~23~~19. The lipid nanoparticle according to claim ~~22~~18, wherein the nucleic acid is a closed-ended DNA (ceDNA).

~~24~~20. The lipid nanoparticle according to ~~any one of claims 20-23,~~ claim 16, wherein the lipid nanoparticle comprises one or more of: ~~further comprising~~

a sterol;

a polyethylene glycol (PEG) or a PEG-lipid conjugate; or

a non-cationic lipid.

~~25~~21. The lipid nanoparticle according to claim ~~24~~20, wherein the sterol is a cholesterol;

the PEG-lipid conjugate is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG);

the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3 -pho sphoethanolamine (DLPE); 1,2-diphytanoyl-

sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.

~~26. The lipid nanoparticle according to any one of claims 20-25, further comprising a polyethylene glycol (PEG) or a PEG-lipid conjugate.~~

~~27. The lipid nanoparticle according to claim 26, wherein the PEG-lipid conjugate is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).~~

~~28. The lipid nanoparticle according to any one of claims 20-27, further comprising a non-cationic lipid.~~

~~29. The lipid nanoparticle according to claim 28, wherein the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-pho sphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg~~

~~sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.~~

~~30. The lipid nanoparticle according to claim 29, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).~~

~~31~~22. The lipid nanoparticle according to claim 20~~30~~, wherein the PEG or PEG-lipid conjugate is present at about 2% to about 4%, at about 2% to about 3.5%, at about about 2.5 to about 3% or at about 3%; and/or

the cholesterol is present at a molar percentage of about 20% to about 40%;

the ionizable lipid is present at a molar percentage of about 80% to about 60%.

~~32. The lipid nanoparticle according to claim 31, wherein the PEG or PEG-lipid conjugate is present at about 2% to about 3.5%.~~

~~33. The lipid nanoparticle according to claim 32, wherein the PEG or PEG-lipid conjugate is present at about 2.5 to about 3%.~~

~~34. The lipid nanoparticle according to claim 33, wherein the PEG or PEG-lipid conjugate is present at about 3%.~~

~~35. The lipid nanoparticle according to any one of claims 29-34, wherein the cholesterol is present at a molar percentage of about 20% to about 40%, and wherein the ionizable lipid is present at a molar percentage of about 80% to about 60%.~~

~~36~~23. The lipid nanoparticle according to claim 22~~35~~, wherein the cholesterol is present at a molar percentage of about 40%, and wherein the ionizable lipid is present at a molar percentage of about 50%.

~~37. The lipid nanoparticle according to any one of claims 19-23, further comprising a cholesterol, a PEG or PEG-lipid conjugate, and a non-cationic lipid.~~

~~38. The lipid nanoparticle according to claim 37, wherein the PEG or PEG-lipid conjugate is present at about 2% to about 4%.~~

~~39. The lipid nanoparticle according to claim 38, wherein the PEG or PEG-lipid conjugate is present at about 2% to about 3.5%.~~

~~40. The lipid nanoparticle according to claim 39, wherein the PEG or PEG-lipid conjugate is present at about 2.5 to about 3%.~~

~~41. The lipid nanoparticle according to claim 40, wherein the PEG or PEG-lipid conjugate is present at about 3%.~~

~~42. The lipid nanoparticle according to any one of claims 36-41, wherein the cholesterol is present at a molar percentage of about 30% to about 50%.~~

~~43~~24. The lipid nanoparticle according to ~~any one of~~ claim 23~~claims 36-41~~, wherein~~:~~ ~~-~~the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%~~; and/or~~

~~the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%~~.

~~44. The lipid nanoparticle according to any one of claims 36-41, wherein the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%.~~

~~45. The lipid nanoparticle according to any one of claims 36-41, wherein the cholesterol is present at a molar percentage of about 40%, the ionizable lipid is present at a molar percentage of about 52.5%, the non-cationic lipid is present at a molar percentage of about 7.5%, and wherein the PEG or PEG-lipid conjugate is present at about 3%.~~

~~46~~25. The lipid nanoparticle according to claim 16~~any one of claims 20-45~~, further comprising dexamethasone palmitate.

~~47~~26. The lipid nanoparticle according to claim 16~~any one of claims 20-46~~, wherein the nanoparticle has a diameter ranging from about 50 nm to about 110 nm~~; or the~~

nanoparticle is less than about 100 nm in size; or the nanoparticle is less than about 70 nm in size.

~~48. The lipid nanoparticle according to any one of claims 20-46, wherein the nanoparticle is less than about 100 nm in size.~~

~~49. The lipid nanoparticle according to claim 48, wherein the particle is less than about 70 nm in size.~~

~~50. The lipid nanoparticle according to claim 49, wherein the particle is less than about 60 nm in size.~~

~~51~~27. The lipid nanoparticle according to claim ~~23~~19, wherein the nanoparticle has a total lipid to ceDNA ratio of about 10:1; or about 20:1; or about 30:1; or about 40:1.

~~52. The lipid nanoparticle according to any one of claims 23, wherein the particle has a total lipid to ceDNA ratio of about 20:1.~~

~~53. The lipid nanoparticle according to any one of claims 23, wherein the particle has a total lipid to ceDNA ratio of about 30:1.~~

~~54. The lipid nanoparticle according to any one of claims 23, wherein the particle has a total lipid to ceDNA ratio of about 40:1.~~

~~55~~28. The lipid nanoparticle according to claim 16~~any one of claims 20-54~~, further comprising a tissue specific targeting moiety.

~~56~~29. The lipid nanoparticle according to claim ~~55~~28, wherein the tissue specific targeting moiety is N-acetylgalactosamine (GalNAc) containing moiety (*e.g.*, GalNAC-PEG-lipid conjugate) and is present in the nanoparticle at a molar percentage of about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, or about 0.1% of the total lipid.

~~57. The lipid nanoparticle according to claim 56, wherein the GalNAc containing moiety is present in the particle at a molar percentage of about 0.5% of the total lipid.~~

~~58. The lipid nanoparticle according to any one of claims 20-57, further comprising about 10 mM to about 30 mM malic acid.~~

~~59. The lipid nanoparticle according to claim 58, comprising about 20 mM malic acid.~~

~~60. The lipid nanoparticle according to any one of claims 20-59, further comprising about 30 mM to about 50 mM NaCl.~~

~~61. The lipid nanoparticle according to claim 60, further comprising about 40 mM NaCl.~~

~~62. The lipid nanoparticle according to any one of claims 20-61, further comprising about 20 mM to about 100 mM $MgCl_2$.~~

~~63~~30. The lipid nanoparticle according to claim ~~23~~19, wherein the ceDNA is a closed-ended linear duplex DNA.

~~64. The lipid nanoparticle according to claim 23, wherein the ceDNA comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene.~~

~~65. The lipid nanoparticle according to claim 64, wherein the expression cassette comprises a polyadenylation sequence.~~

~~66. The lipid nanoparticle according to any one of claims 63-65, wherein the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.~~

~~67. The lipid nanoparticle according to claim 66, wherein the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.~~

~~68. The lipid nanoparticle according to claim 66, wherein the expression cassette is connected to an ITR at 3' end (3' ITR).~~

~~69. The lipid nanoparticle according to claim 66, wherein the expression cassette is connected to an ITR at 5' end (5' ITR).~~

~~70. The lipid nanoparticle according to claim 66, wherein at least one of 5' ITR and 3' ITR is a wild-type AAV ITR.~~

~~71. The lipid nanoparticle according to claim 66, wherein at least one of 5' ITR and 3' ITR is a modified ITR.~~

~~72. The lipid nanoparticle according to claim 66, wherein the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.~~

~~73. The lipid nanoparticle according to claim 66, wherein the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette.~~

~~74. The lipid nanoparticle according to claim 72 or claim 73, wherein the spacer sequence is at least 5 base pairs long in length.~~

~~75. The lipid nanoparticle according to claim 74, wherein the spacer sequence is 5 to 100 base pairs long in length.~~

~~76. The lipid nanoparticle according to claim 74, wherein the spacer sequence is 5 to 500 base pairs long in length.~~

~~77. The lipid nanoparticle according to any one of claims 20-76, wherein the ceDNA has a nick or a gap.~~

~~78. The lipid nanoparticle according to claim 66, wherein the ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, a wild-type ITR from a parvovirus.~~

~~79. The lipid nanoparticle according to claim 78, wherein said AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.~~

~~80. The lipid nanoparticle according to claim 66, wherein the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR.~~

~~81. The lipid nanoparticle according to claim 66, wherein the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette, optionally wherein the two mutant ITRs are symmetric mutants.~~

~~82~~31. The lipid nanoparticle according to claim ~~23~~19, wherein the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministering DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA.

~~83~~32. A pharmaceutical composition comprising the lipid nanoparticle according to claim 16~~any one of claims 20-82~~ and a pharmaceutically acceptable excipient.

~~84~~33. Use of a lipid nanoparticle according to any one of claims 16-31, or an effective amount of the pharmaceutical composition according to claim 32, in the manufacture of a medicament for~~A method of~~ treating a genetic disorder in a subject~~, the method comprising administering to the subject an effective amount of the lipid nanoparticle according to any one of claims 20-80, or an effective amount of the pharmaceutical composition according to claim 83~~.

~~85. The method of claim 84, wherein the subject is a human.~~

~~86~~34. The use of claim 33~~method any one of claims 84 or 85~~, wherein the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria,

inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, mucopolysaccharide storage diseases (e.g., Hurler syndrome (MPS Type I), Scheie syndrome (MPS Type I S), Hurler-Scheie syndrome (MPS Type I H-S), Hunter syndrome (MPS Type II), Sanfilippo Types A, B, C, and D (MPS Types III A, B, C, and D), Morquio Types A and B (MPS IVA and MPS IVB), Maroteaux-Lamy syndrome (MPS Type VI), Sly syndrome (MPS Type VII), hyaluronidase deficiency (MPS Type IX)), Niemann-Pick Disease Types A/B, C1 and C2, Fabry disease, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease Types I and II (Pompe disease), Gaucher disease Types I, II and III, Fabry disease, cystinosis, Batten disease, Aspartylglucosaminuria, Salla disease, Danon disease (LAMP-2 deficiency), Lysosomal Acid Lipase (LAL) deficiency, neuronal ceroid lipofuscinoses (CLN1-8, INCL, and LINCL), sphingolipidoses, galactosialidosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, spinocerebellar ataxia, spinal muscular atrophy, Friedreich's ataxia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophies (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2) and Cathepsin A deficiency.

~~87~~35. The use~~method~~ of claim ~~86~~34, wherein the genetic disorder is selected from the group consisting of: Leber congenital amaurosis (LCA), Niemann-Pick disease, Stargardt macular dystrophy, glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I), Pompe disease (glycogen storage disease type II), hemophilia A (Factor VIII deficiency), hemophilia B (Factor IX deficiency), hunter syndrome (Mucopolysaccharidosis II), cystic fibrosis, dystrophic epidermolysis bullosa (DEB), phenylketonuria (PKU), progressive familial intrahepatic cholestasis (PFIC), Wilson disease, and Gaucher disease Type I, II or III.

~~88. The method of claim 87, wherein the LCA is LCA10.~~

~~89. The method of claim 86, wherein the genetic disorder is Niemann-Pick disease.~~

~~90. The method of claim 86, wherein the genetic disorder is Stargardt macular dystrophy.~~

~~91. The method of claim 86, wherein the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II).~~

~~92. The method of claim 86, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).~~

~~93. The method of claim 86, wherein the genetic disorder is hemophilia B (Factor IX deficiency).~~

~~94. The method of claim 86, wherein the genetic disorder is hunter syndrome (Mucopolysaccharidosis II).~~

~~95. The method of claim 86, wherein the genetic disorder is cystic fibrosis.~~

~~96. The method of claim 86, wherein the genetic disorder is dystrophic epidermolysis bullosa (DEB).~~

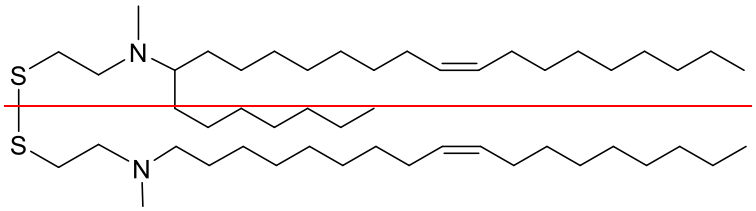
~~97. The method of claim 86, wherein the genetic disorder is phenylketonuria (PKU).~~

~~98. The method of claim 86, wherein the genetic disorder is progressive familial intrahepatic cholestasis (PFIC).~~

~~99. The method of claim 86, wherein the genetic disorder is Wilson disease.~~

~~100. The method of claim 86, wherein the genetic disorder is Gaucher disease Type I, II or III.~~

~~101. The ionizable lipid according to Claim 1, wherein the ionizable lipid is (Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfanyl)ethyl)pentacos-16-en-8-amine whose formula is set forth below:~~



~~.~~

36. The lipid nanoparticle according to any one of claims 16-31, or an effective amount of the pharmaceutical composition according to claim 32, for use in therapy.