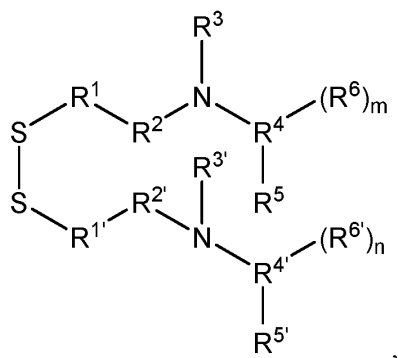


CLAIMS

What is Claimed is:

- 5 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;
- 10 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;
- 15 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,
- 20 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;
- 25 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.

2. The ionizable lipid according to claim 1, wherein the linear or branched C₁₋₃ alkylene represented by R¹ or R^{1'}, the linear or branched C₁₋₆ alkylene represented by R² or R^{2'}, and the optionally substituted linear or branched C₁₋₆ alkyl are each optionally substituted with one or more halo and cyano groups.

3. The ionizable lipid according to claim 1, wherein R² and R^{2'} are each independently C₁₋₃ alkylene.

4. The ionizable lipid according to claim 3, wherein R¹ and R² taken together are C₁₋₃ alkylene and R^{1'} and R^{2'} taken together are C₁₋₃ alkylene.

5. The ionizable lipid according to claim 4, wherein R¹ and R² taken together are ethylene and R^{1'} and R^{2'} taken together are ethylene.

6. The ionizable lipid according to claim 1, wherein R³ and R^{3'} are each independently optionally substituted C₁₋₃ alkyl.

7. The ionizable lipid according to claim 6, wherein R³ and R^{3'} are each methyl.

8. The ionizable lipid according to claim 1, wherein R⁴ and R^{4'} are each -CH.

9. The ionizable lipid according to claim 1, wherein R² is optionally substituted branched C₁₋₆ alkylene; and wherein R² and R³, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

10. The ionizable lipid according to claim 1, wherein R^{2'} is optionally substituted branched C₁₋₆ alkylene; and wherein R^{2'} and R^{3'}, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

11. The ionizable lipid according to claim 1, wherein R⁴ is -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a and R^a is C₁₋₃ alkyl; and wherein R³ and R⁴, taken together with their intervening N atom, form a 5- or 6-membered heterocyclyl.

12. 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.

5

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

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

10

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

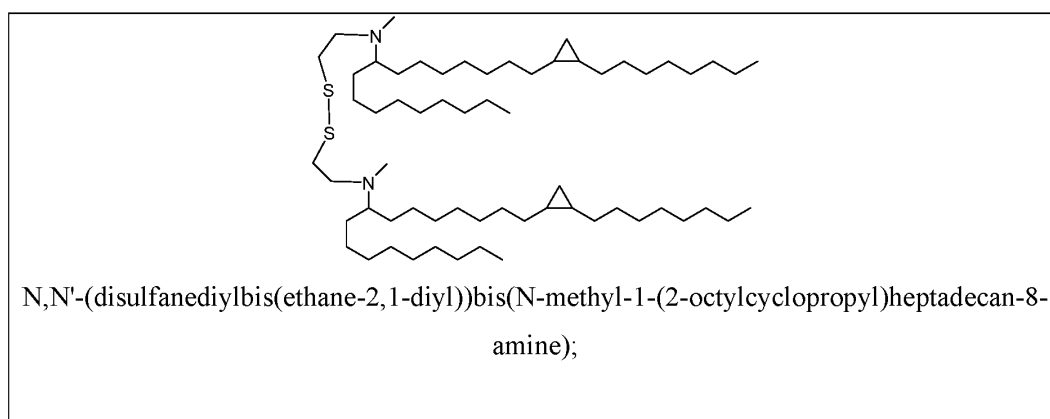
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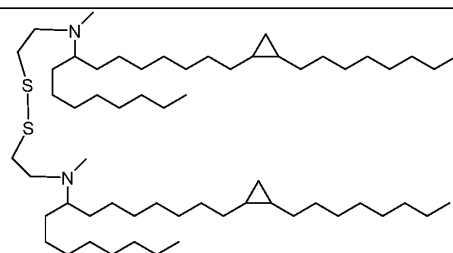
16. The ionizable lipid according to claim 15, wherein the C_{3-10} cycloalkylene is cyclopropylene.

17. The ionizable lipid according to claim 15 or claim 16, wherein m and n are each 3.

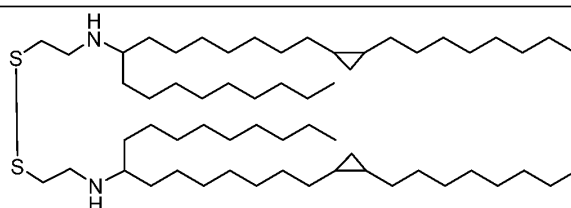
20

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

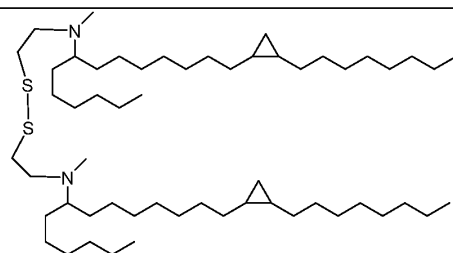




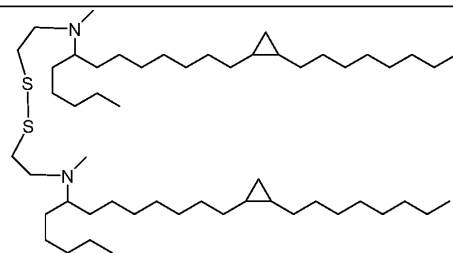
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)hexadecan-8-amine);



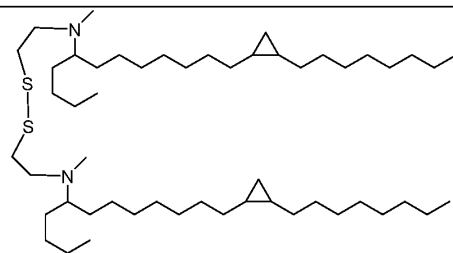
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(1-(2-octylcyclopropyl)heptadecan-8-amine);

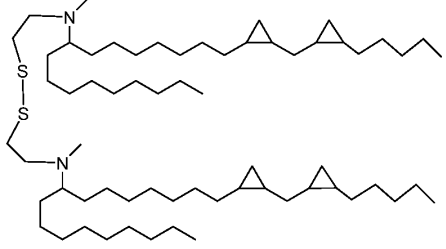
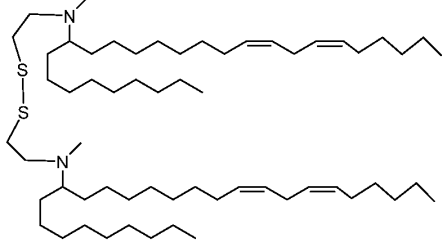
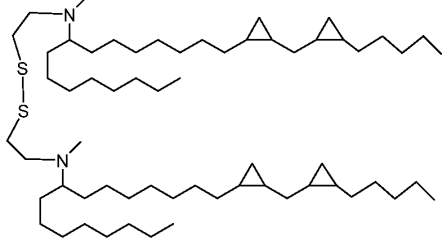
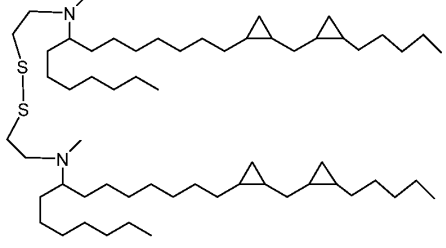


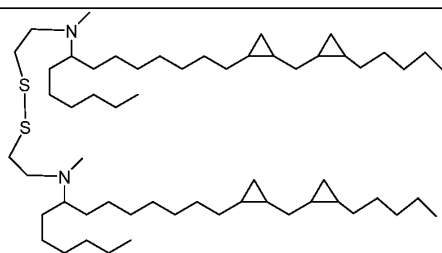
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-14-(2-octylcyclopropyl)tetradecan-7-amine);



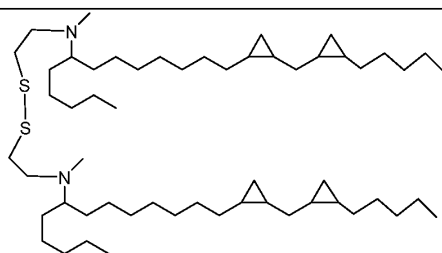
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-13-(2-octylcyclopropyl)tridecan-6-amine);



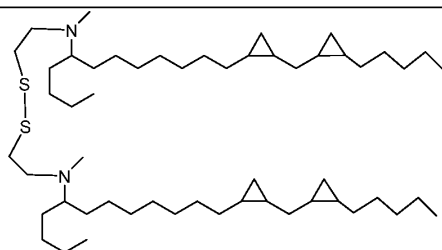
N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyl-12-(2-octylcyclopropyl)dodecan-5-amine);
 N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyl-1-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)heptadecan-8-amine);
 (18Z,18'Z,21Z,21'Z)-N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methylheptacos-18,21-dien-10-amine);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



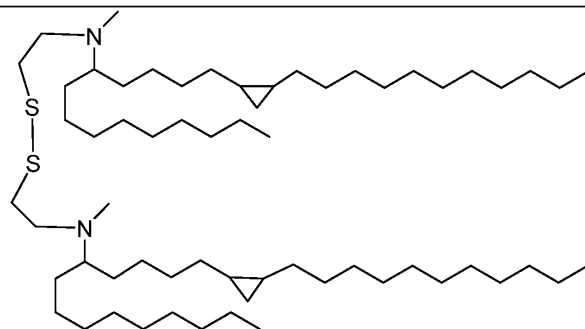
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-14-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)tetradecan-7-amine);



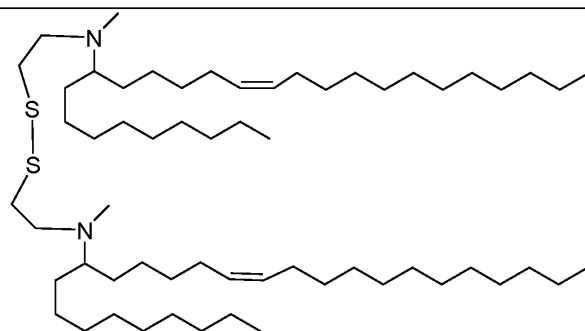
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-13-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)tridecan-6-amine);



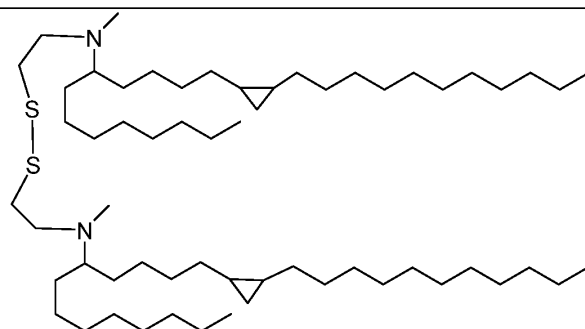
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-12-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)dodecan-5-amine);



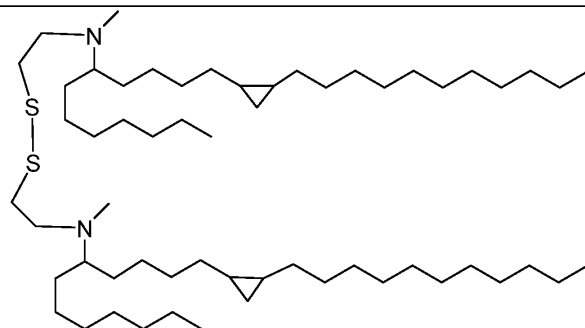
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)tetradecan-5-amine);



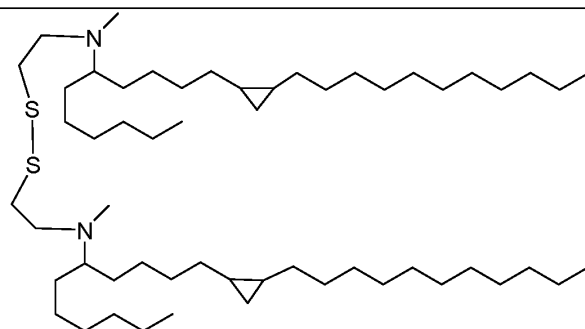
(15Z,15'Z)-N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methylheptacos-15-en-10-amine);



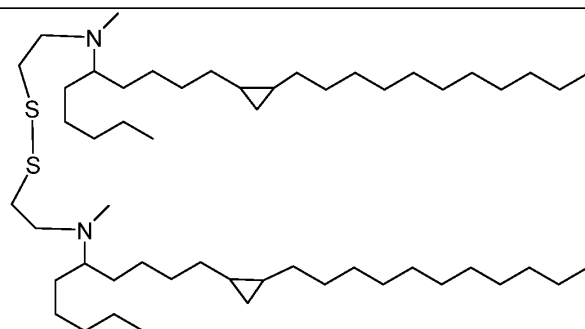
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)tridecan-5-amine);



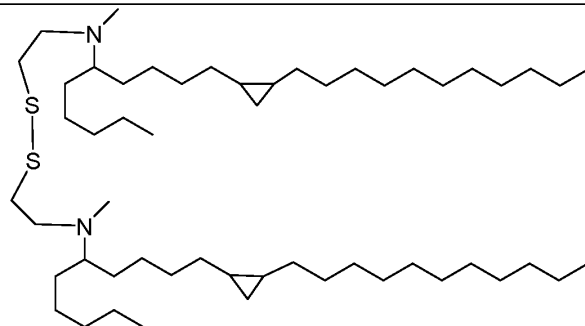
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)dodecan-5-amine);



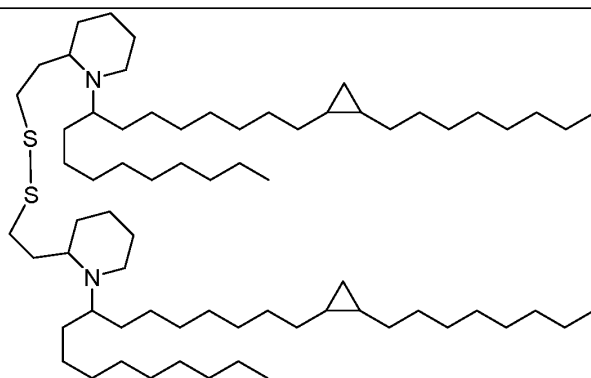
N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)undecan-5-amine);



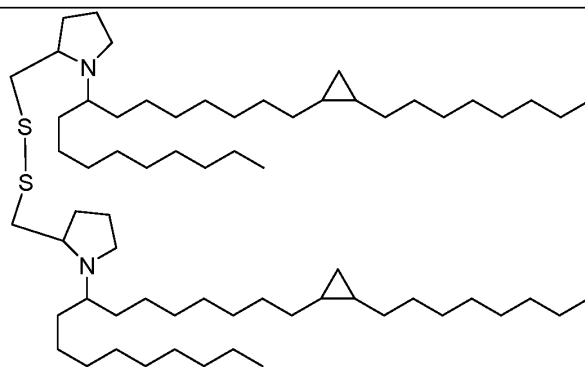
N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)decan-5-amine);



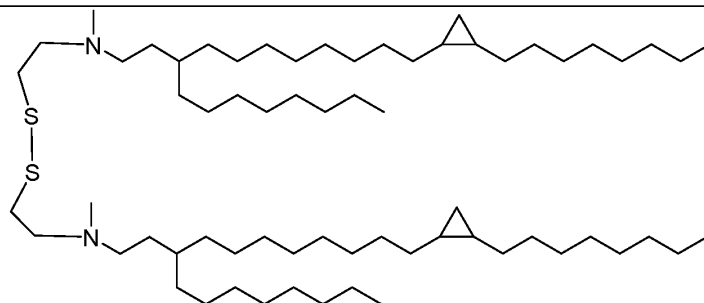
N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyl-1-(2-undecylcyclopropyl)decan-5-amine);



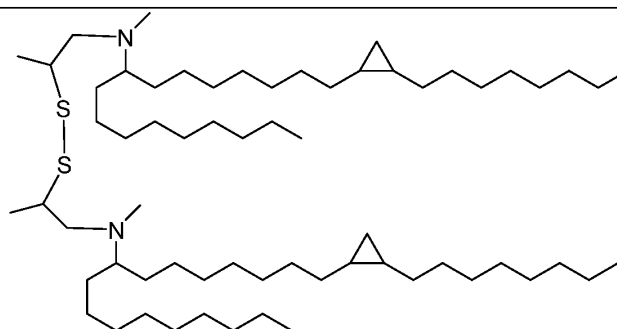
1,2-bis(2-(1-(1-(2-octylcyclopropyl)heptadecan-8-yl)piperidin-2-yl)ethyl)disulfane;



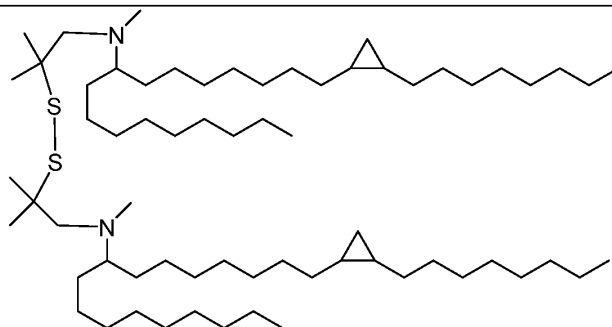
1,2-bis((1-(1-(2-octylcyclopropyl)heptadecan-8-yl)pyrrolidin-2-yl)methyl)disulfane;



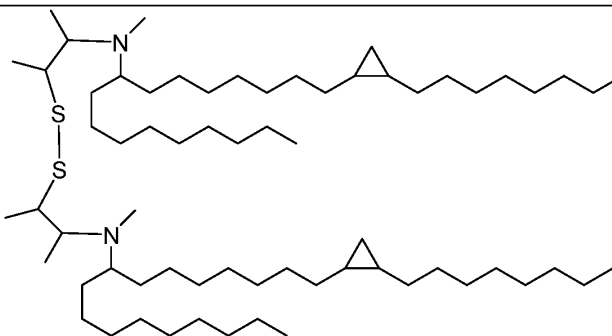
N,N'-(disulfanediy)bis(ethane-2,1-diyl))bis(N-methyl-3-octyl-11-(2-octylcyclopropyl)undecan-1-amine);



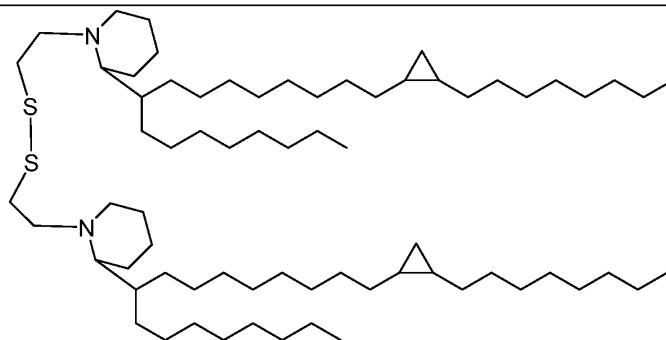
N,N'-(disulfanediylbis(propane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-9-amine);



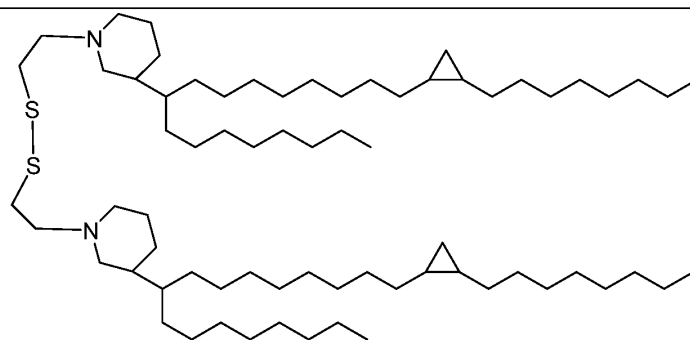
N,N'-(disulfanediylbis(2-methylpropane-2,1-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-8-amine);



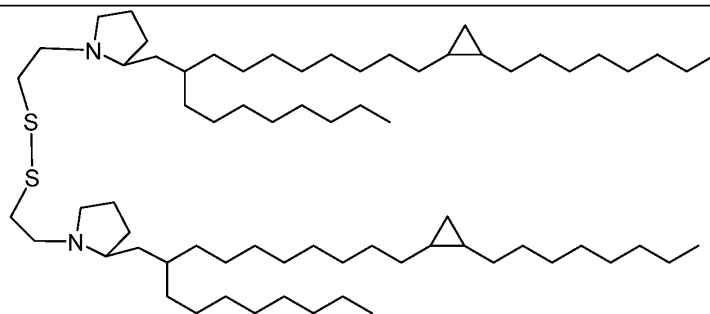
N,N'-(disulfanediylbis(butane-3,2-diyl))bis(N-methyl-1-(2-octylcyclopropyl)heptadecan-8-amine);



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



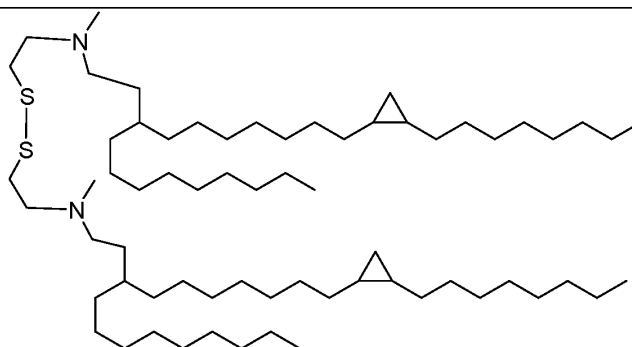
1,2-bis(2-(3-(1-(2-octylcyclopropyl)heptadecan-9-yl)piperidin-1-yl)ethyl)disulfane; and



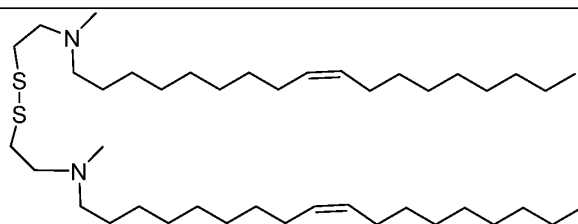
1,2-bis(2-(2-(2-octyl-10-(2-octylcyclopropyl)decyl)pyrrolidin-1-yl)ethyl)disulfane;

or a pharmaceutically acceptable salt of any of the foregoing.

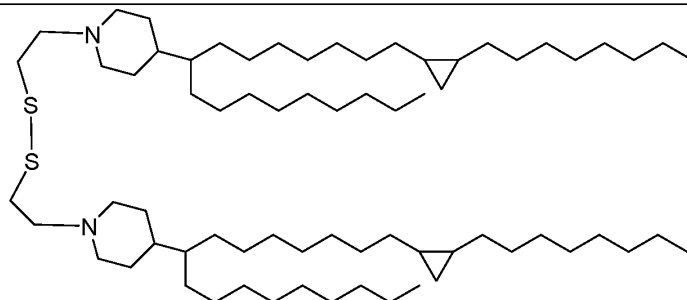
19. The ionizable lipid according to Claim 1, wherein the ionizable lipid is selected from:



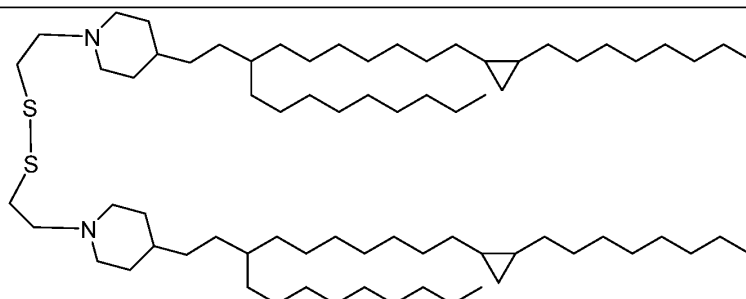
N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N-methyl-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine);



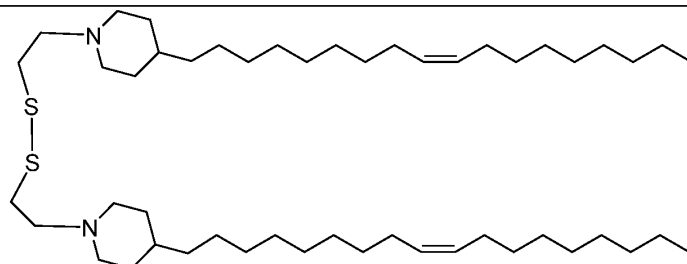
(9Z,9'Z)-N,N'-(disulfanediylobis(ethane-2,1-diyl))bis(N-methyloctadec-9-en-1-amine);



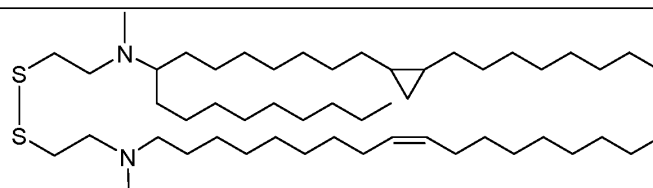
1,2-bis(2-(4-(1-(2-octylcyclopropyl)heptadecan-8-yl)piperidin-1-yl)ethyl)disulfane;



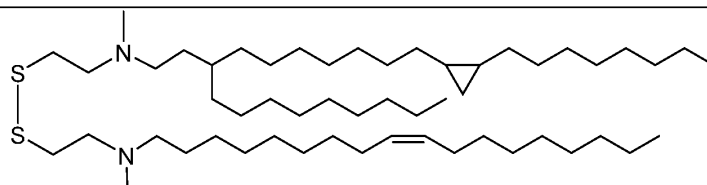
1,2-bis(2-(4-(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)piperidin-1-yl)ethyl)disulfane;



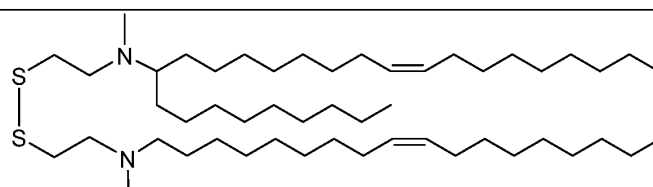
1,2-bis(2-(4-((Z)-octadec-9-en-1-yl)piperidin-1-yl)ethyl)disulfane;



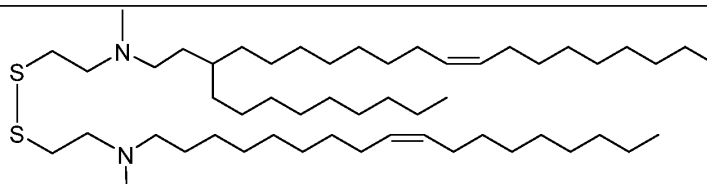
(Z)-N-methyl-N-(2-((2-(methyl(1-(2-octylcyclopropyl)heptadecan-8-yl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;



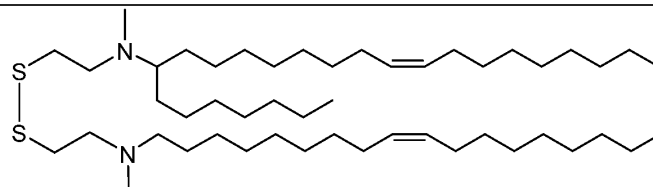
(Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;



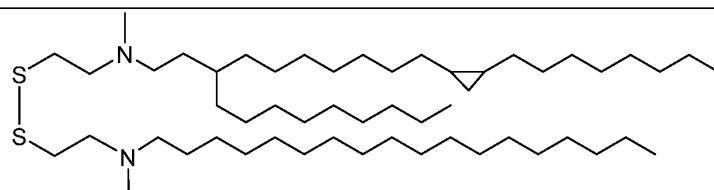
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)heptacos-18-en-10-amine;



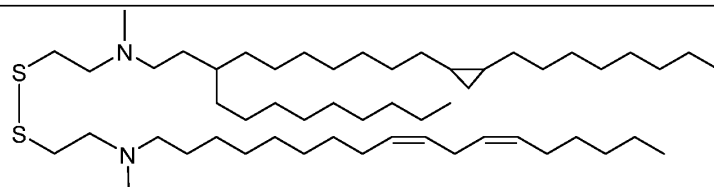
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)-3-nonylicos-11-en-1-amine;



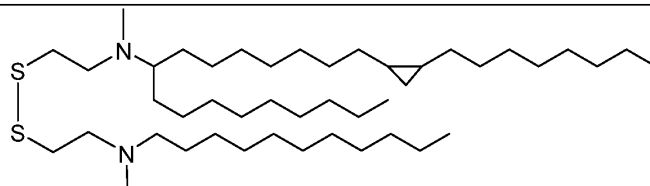
(Z)-N-methyl-N-(2-((2-(methyl((Z)-octadec-9-en-1-yl)amino)ethyl)disulfaneyl)ethyl)pentacos-16-en-8-amine;



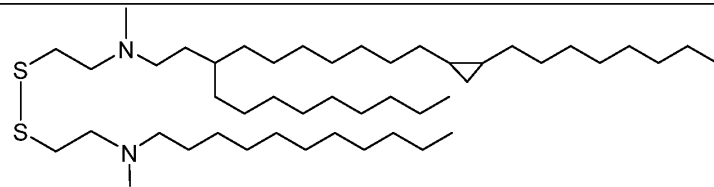
N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;



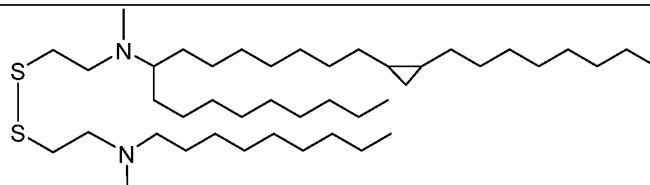
(9Z,12Z)-N-methyl-N-(2-((2-(methyl(3-(7-(2-octylcyclopropyl)heptyl)dodecyl)amino)ethyl)disulfaneyl)ethyl)octadeca-9,12-dien-1-amine;



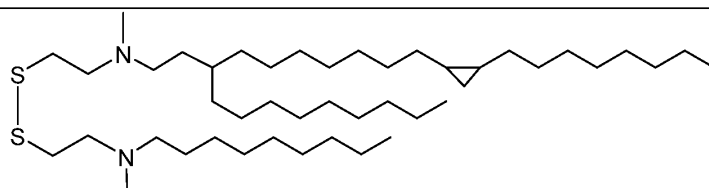
N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;



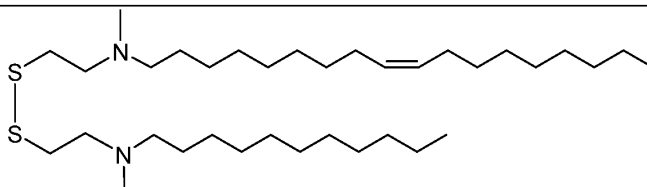
N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;



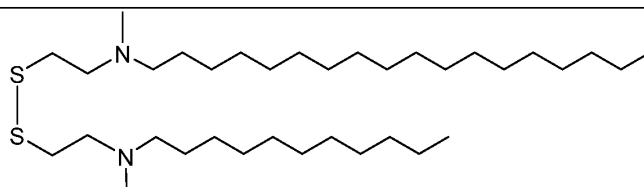
N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-1-(2-octylcyclopropyl)heptadecan-8-amine;



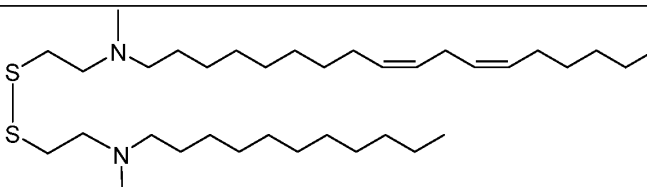
N-methyl-N-(2-((2-(methyl(nonyl)amino)ethyl)disulfaneyl)ethyl)-3-(7-(2-octylcyclopropyl)heptyl)dodecan-1-amine;



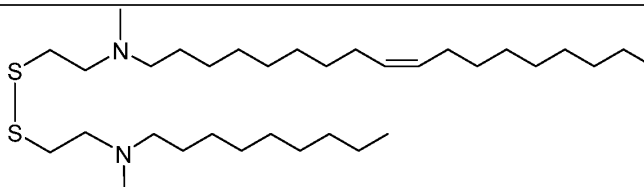
(Z)-N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadec-9-en-1-amine;



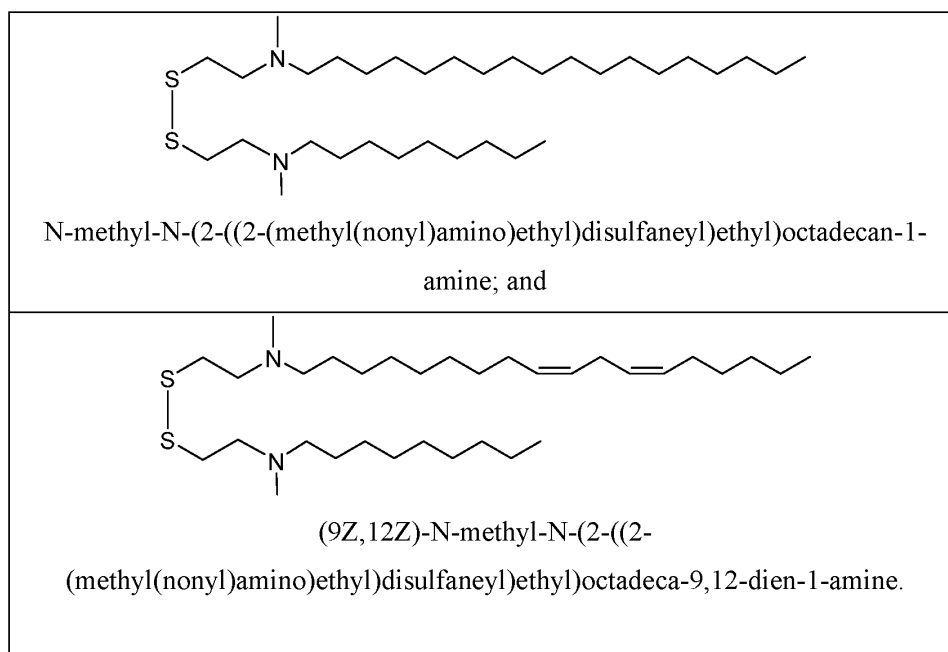
N-methyl-N-(2-((2-(methyl(undecyl)amino)ethyl)disulfaneyl)ethyl)octadecan-1-amine;



(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;



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

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

22. The lipid nanoparticle according to claim 20 or claim 21, 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.

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

24. The lipid nanoparticle according to any one of claims 20-23, further comprising a sterol.

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

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 1-(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-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg

sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, or mixtures thereof.

5 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).

10 31. The lipid nanoparticle according to claim 30, wherein the PEG or PEG-lipid conjugate is present at about 2% to about 4%.

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

15 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%.

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 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%.

25 36. The lipid nanoparticle according to claim 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%.

30 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. The lipid nanoparticle according to any one of claims 36-41, wherein the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%.

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. The lipid nanoparticle according to any one of claims 20-45, further comprising dexamethasone palmitate.

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

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.

5 51. The lipid nanoparticle according to claim 23, wherein the particle has a total lipid to ceDNA ratio of about 10:1.

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.

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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
15 has a total lipid to ceDNA ratio of about 40:1.

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

20 56. The lipid nanoparticle according to claim 55, wherein the tissue specific targeting moiety is N-acetylgalactosamine (GalNAc) containing moiety (*e.g.*, GalNAC-PEG-lipid conjugate) and is present in the particle 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
25 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.

30 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.

5 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 MgCl₂.

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63. The lipid nanoparticle according to claim 23, 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.

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

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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.

25 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).

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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.

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82. The lipid nanoparticle according to claim 23, 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.

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

84. 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.

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

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86. The 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

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Leukodystrophy, Krabbe disease, Mucopolidosis 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
5 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
10 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.

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87. The method of claim 86, wherein the genetic disorder is Leber congenital amaurosis (LCA).

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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.

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

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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
5 (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
10 epidermolysis bullosa (DEB).

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

15 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.

20 100. 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)disulfaneyl)ethyl)pentacos-16-en-8-amine whose formula is set forth below:

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