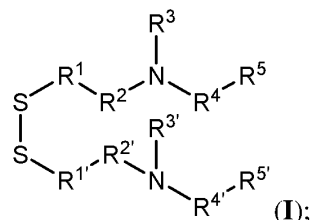


CLAIMS

What is Claimed is:

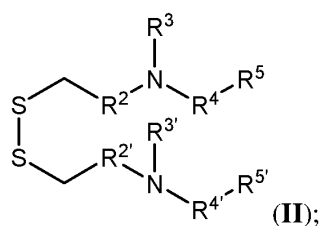
- 5 1. An ionizable lipid of the Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

- R^1 and $R^{1'}$ are each independently (C_1-C_6) alkylene optionally substituted with one or more groups selected from R^a ;
- 10 R^2 and $R^{2'}$ are each independently (C_1-C_2) alkylene;
- R^3 and $R^{3'}$ are each independently (C_1-C_6) alkyl optionally substituted with one or more groups selected from R^b ;
- or alternatively, R^2 and R^3 and/or $R^{2'}$ and $R^{3'}$ are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;
- 15 R^4 and $R^{4'}$ are each a (C_2-C_6) alkylene interrupted by $-C(O)O-$;
- R^5 and $R^{5'}$ are each independently a (C_2-C_{30}) alkyl or (C_2-C_{30}) alkenyl, each of which are optionally interrupted with $-C(O)O-$ or (C_3-C_6) cycloalkyl; and
- R^a and R^b are each halo or cyano.
- 20 2. The ionizable lipid of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^1 and $R^{1'}$ are each independently (C_1-C_6) alkylene.
3. The ionizable lipid of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein R^1 and $R^{1'}$ are each independently (C_1-C_3) alkylene.
- 25

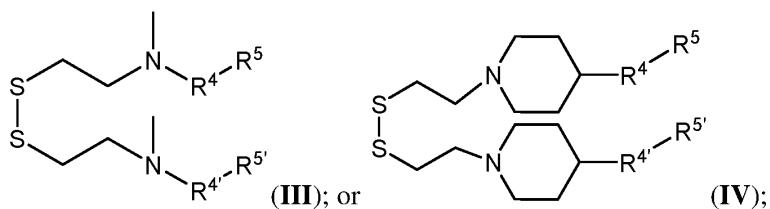
4. The ionizable lipid of any one of claims 1 to 3, wherein the lipid is of the Formula (II):



or a pharmaceutically acceptable salt thereof.

5

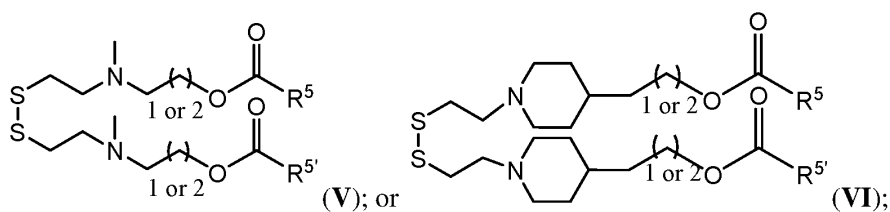
5. The ionizable lipid of any one of claims 1 to 4, wherein the lipid is of the Formula (III) or (IV):



or a pharmaceutically acceptable salt thereof.

10

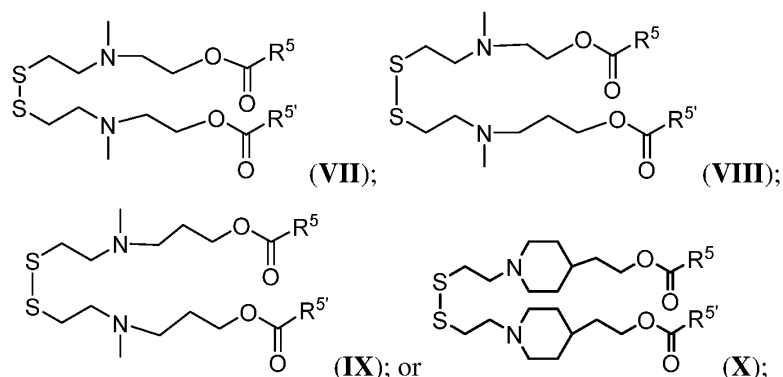
6. The ionizable lipid of any one of claims 1 to 5, wherein the lipid is of the Formula (V) or (VI):



or a pharmaceutically acceptable salt thereof.

15

7. The ionizable lipid of any one of claims 1 to 6, wherein the lipid is of the Formula (VII) or (VIII):



5 or a pharmaceutically acceptable salt thereof.

8. The ionizable lipid of any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₆-C₂₆)alkyl or (C₆-C₂₆)alkenyl, each of which are optionally interrupted with -C(O)O- or (C₃-C₆)cycloalkyl.

10

9. The ionizable lipid of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₆-C₂₄)alkyl or (C₆-C₂₄)alkenyl, each of which are optionally interrupted with -C(O)O- or cyclopropyl.

15 10. The ionizable lipid of any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₈-C₂₄)alkyl or (C₈-C₂₄)alkenyl, wherein said (C₈-C₂₄)alkyl is optionally interrupted with -C(O)O- or cyclopropyl.

20 11. The ionizable lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₈-C₁₀)alkyl.

12. The ionizable lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₁₄-C₁₆)alkyl interrupted with cyclopropyl.

25 13. The ionizable lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C₁₀-C₂₄)alkyl interrupted with -C(O)O-.

14. The ionizable lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^5 is a $(C_{16}-C_{18})$ alkenyl.
15. The ionizable lipid of any one of claims 1 to 14, or a pharmaceutically acceptable salt thereof, wherein R^5 is $-(CH_2)_3C(O)O(CH_2)_8CH_3$, $-(CH_2)_5C(O)O(CH_2)_8CH_3$, $-(CH_2)_7C(O)O(CH_2)_8CH_3$, $-(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $-(CH_2)_7-C_3H_6-(CH_2)_7CH_3$, $-(CH_2)_7CH_3$, $-(CH_2)_9CH_3$, $-(CH_2)_{16}CH_3$, $-(CH_2)_7CH=CH(CH_2)_7CH_3$, or $-(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$.
16. The ionizable lipid of any one of claims 1 to 15, or a pharmaceutically acceptable salt thereof, wherein R^5 is a $(C_{15}-C_{28})$ alkyl interrupted with $-C(O)O-$.
17. The ionizable lipid of any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, wherein R^5 is a $(C_{20}-C_{26})$ alkyl interrupted with $-C(O)O-$.
18. The ionizable lipid of any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof, wherein R^5 is a $(C_{22}-C_{24})$ alkyl interrupted with $-C(O)O-$.
19. The ionizable lipid of any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, wherein R^5 is $-(CH_2)_5C(O)OCH[(CH_2)_7CH_3]_2$, $-(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $-(CH_2)_5C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$, or $-(CH_2)_7C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$.
20. A lipid nanoparticle (LNP) comprising the ionizable lipid of any one of claims 1 to 19, or a pharmaceutically acceptable salt thereof; and a nucleic acid.
21. The lipid nanoparticle of claim 20, wherein the nucleic acid is encapsulated in the lipid.
22. The lipid nanoparticle of 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, ccDNA, ministring, doggyboneTM, 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. The lipid nanoparticle of claim 22, wherein the nucleic acid is a closed-ended DNA
5 (ceDNA).

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

25. The lipid nanoparticle of claim 24, wherein the sterol is a cholesterol or beta-
10 sitosterol.

26. The lipid nanoparticle of any one of claims 20 to 25, further comprising a PEGylated lipid.

15 27. The lipid nanoparticle of claim 26, wherein the PEGylated lipid is 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG).

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

29. The lipid nanoparticle of 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),
25 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),
30 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), dierycoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin,
- 5 lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, and dilinoleoylphosphatidylcholine, and mixtures thereof.
- 10 30. The lipid nanoparticle of claim 29, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).
31. The lipid nanoparticle of claim 30, wherein the PEGylated lipid is present at a molar
- 15 percentage of about 1.5% to about 4%.
32. The lipid nanoparticle of claim 31, wherein the PEGylated lipid is present at a molar percentage of about 2% to about 3%.
- 20 33. The lipid nanoparticle of claim 32, wherein the PEGylated lipid is present at a molar percentage of about 2.5 to about 3%.
34. The lipid nanoparticle of claim 33, wherein the PEGylated lipid is present at a molar percentage of about 3%.
- 25 35. The lipid nanoparticle of claim 31, wherein the PEGylated lipid is present at a molar percentage of about 4%.
36. The lipid nanoparticle of any one of claims 25 to 35, wherein the sterol is present at a
- 30 molar percentage of about 20% to about 40%, and wherein the lipid is present at a molar percentage of about 80% to about 60%.
37. The lipid nanoparticle of claim 36, wherein the sterol is present at a molar percentage of about 40%, and wherein the lipid is present at a molar percentage of about 50%.

38. The lipid nanoparticle of any one of claims 20 to 23, further comprising a cholesterol, a PEGylated lipid, and a non-cationic lipid.
39. The lipid nanoparticle of claim 38, wherein the PEGylated lipid is present at a molar percentage of about 1.5% to about 4%.
40. The lipid nanoparticle of claim 39, wherein the PEGylated lipid is present at about a molar percentage of 2% to about 3%.
41. The lipid nanoparticle of claim 40, wherein the PEGylated lipid is present at about a molar percentage of 2.5% to about 3%.
42. The lipid nanoparticle of claim 41, wherein the PEGylated lipid is present at a molar percentage of about 3%.
43. The lipid nanoparticle of claim 38, wherein the cholesterol is present at a molar percentage of about 30% to about 50%.
44. The lipid nanoparticle of claim 43, wherein the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%.
45. The lipid nanoparticle of any one of claims 38 to 44, wherein the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%.
46. The lipid nanoparticle of any one of claims 38 to 45, wherein the cholesterol is present at a molar percentage of about 40%, the 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 PEGylated lipid is present at a molar percentage of about 3%.
47. The lipid nanoparticle of any one of claims 20 to 46, further comprising a tissue-specific targeting ligand.
48. The lipid nanoparticle of claim 47, wherein the tissue-specific targeting ligand is conjugated to a PEGylated lipid and is N-acetylgalactosamine (GalNAc) or a derivative

thereof selected from mono-antennary GalNAc, tri-antennary GalNAc, and tetra-antennary GalNAc.

49. The lipid nanoparticle of claim 48, wherein the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto 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%.

50. The lipid nanoparticle of any one of claims 20 to 49, further comprising dexamethasone palmitate.

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

52. The lipid nanoparticle of any one of claims 20 to 51, wherein the nanoparticle is less than about 100 nm in size.

53. The lipid nanoparticle of claim 52, wherein the particle is less than about 75 nm in size.

54. The lipid nanoparticle of claim 53, wherein the particle is less than about 70 nm in size.

55. The lipid nanoparticle of claim 54, wherein the particle is less than about 65 nm in size.

56. The lipid nanoparticle of any one of claims 1 to 55, wherein the particle has a total lipid to ceDNA ratio of about 10:1.

57. The lipid nanoparticle of claim 56, wherein the particle has a total lipid to ceDNA ratio of about 20:1.

58. The lipid nanoparticle of claim 57, wherein the particle has a total lipid to ceDNA ratio of about 30:1.
59. The lipid nanoparticle of claim 58, wherein the particle has a total lipid to ceDNA ratio of about 40:1.
60. The lipid nanoparticle of any one of claims 20 to 59, further comprising about 10 mM to about 30 mM malic acid.
61. The lipid nanoparticle of claim 60, comprising about 20 mM malic acid.
62. The lipid nanoparticle of any one of claims 20 to 61, further comprising about 30 mM to about 50 mM NaCl.
63. The lipid nanoparticle of claim 62, further comprising about 40 mM NaCl.
64. The lipid nanoparticle of any one of claims 20 to 63, further comprising about 20 mM to about 100 mM MgCl₂.
65. The lipid nanoparticle of claim 23, wherein the ceDNA is a closed-ended linear duplex DNA.
66. The lipid nanoparticle of claim 65, wherein the ceDNA comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene.
67. The lipid nanoparticle of claim 66, wherein the expression cassette comprises a polyadenylation sequence.
68. The lipid nanoparticle of any one of claims 65 to 67, wherein the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette.
69. The lipid nanoparticle of claim 68, wherein the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR.

70. The lipid nanoparticle of claim 68, wherein the expression cassette is connected to an ITR at 3' end (3' ITR).
71. The lipid nanoparticle of claim 68, wherein the expression cassette is connected to an ITR at 5' end (5' ITR).
72. The lipid nanoparticle of claim 68, wherein at least one of 5' ITR and 3' ITR is a wild-type AAV ITR.
73. The lipid nanoparticle of claim 68, wherein at least one of 5' ITR and 3' ITR is a modified ITR.
74. The lipid nanoparticle of claim 68, wherein the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.
75. The lipid nanoparticle of claim 68, wherein the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette.
76. The lipid nanoparticle of claim 74 or claim 75, wherein the spacer sequence is at least 5 base pairs long in length.
77. The lipid nanoparticle of claim 76, wherein the spacer sequence is 5 to 100 base pairs long in length.
78. The lipid nanoparticle of claim 76, wherein the spacer sequence is 5 to 500 base pairs long in length.
79. The lipid nanoparticle of any one of claims 23 to 78, wherein the ceDNA has a nick or a gap.
80. The lipid nanoparticle of claim 68, 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.

81. The lipid nanoparticle according to claim 80, wherein said AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.
- 5 82. The lipid nanoparticle of claim 68, wherein the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR.
83. The lipid nanoparticle of claim 68, wherein the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette, optionally wherein the two mutant ITRs are
10 symmetric mutants.
84. The lipid nanoparticle of 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
15 cassette, or a doggybone™ DNA.
85. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 20 to 84 and a pharmaceutically acceptable excipient.
- 20 86. A pharmaceutical composition comprising the lipid of any one of claims 1 to 19 or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.
87. A method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the lipid nanoparticle of any one of claims
25 20 to 84, or an effective amount of the pharmaceutical composition according to claim 85 or claim 86.
88. The method of claim 87, wherein the subject is a human.
- 30 89. The method claim 87 or claim 88, 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, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, 5

Mucopolipidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease 10 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 15 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 (LCA), Stargardt macular dystrophy (ABCA4), ornithine

transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, 20 progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2), and Cathepsin A deficiency.

90. The method of claim 89, wherein the genetic disorder is Leber congenital amaurosis (LCA) 10.

91. The method of claim 89, wherein the genetic disorder is Stargardt macular dystrophy (ABCA4).

92. The method of claim 89, wherein the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II).

93. The method of claim 89, wherein the genetic disorder is hemophilia A (Factor VIII deficiency).
94. The method of claim 89, wherein the genetic disorder is hemophilia B (Factor IX deficiency).
95. The method of claim 89, wherein the genetic disorder is hunter syndrome (Mucopolysaccharidosis II).
96. The method of claim 89, wherein the genetic disorder is Usher syndrome.
97. The method of claim 89, wherein the genetic disorder is phenylketonuria (PKU).
98. The method of claim 89, wherein the genetic disorder is progressive familial intrahepatic cholestasis (PFIC).
99. The method of claim 89, wherein the genetic disorder is Wilson disease.
100. The method of claim 89, wherein the genetic disorder is Gaucher disease Type I, II or III.