## CLAIMS

1. A method of producing a lipid nanoparticle (LNP) formulation, the method comprising:

mixing a lipid solution comprising an ionizable lipid with an aqueous buffer solution having a pH in a range of from about 4.8 to about 5.5~~comprising a first buffering agent,~~ thereby forming a lipid nanoparticle solution comprising a lipid nanoparticle; and

adding a nucleic acid solution comprising a nucleic acid to the lipid nanoparticle solution thereby forming a lipid nanoparticle (LNP) formulation comprising the lipid nanoparticle associated with the nucleic acid.

2. The method of claim 1, wherein the lipid nanoparticle of the lipid nanoparticle solution is an empty lipid nanoparticle.

3. The method of claim 1 or 2, wherein the lipid nanoparticle of the lipid nanoparticle solution has an average diameter of less than ~~about~~ 150 nm, less than ~~about~~ 125 nm, less than ~~about~~ 100 nm, less than ~~about~~ 90 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, or less than ~~about~~ 30 nm.

4. The method of any one of claims 1-3, wherein the lipid nanoparticle of the lipid nanoparticle solution has an average diameter of ~~about~~ 25 nm to ~~about~~ 125 nm, ~~about~~ 30 nm to ~~about~~ 110 nm, ~~about~~ 35 nm to ~~about~~ 100 nm, ~~about~~ 40 nm to ~~about~~ 90 nm, ~~about~~ 45 nm to ~~about~~ 80 nm, or ~~about~~ 50 nm to ~~about~~ 70 nm.

5. The method of any one of claims 1-4, wherein the aqueous buffer solution has a pH in a range of ~~about 4.5 to about 6.5, about 4.6 to about 6.0, about 4.7 to about 5.75, about 4.8 to about 5.5, or about~~ 4.9 to ~~about~~ 5.25.

6. The method of any one of claims 1-5, wherein the lipid solution has a pH in a range of ~~about~~ 7.0 to ~~about~~ 8.0, ~~about~~ 7.1 to ~~about~~ 7.8, ~~about~~ 7.2 to ~~about~~ 7.6, or ~~about~~ 7.3 to ~~about~~ 7.5.

7. The method of any one of claims 1-6, wherein the nucleic acid solution has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.5, ~~about~~ 4.8 to ~~about~~ 6.25, ~~about~~ 4.8 to ~~about~~ 6.0, ~~about~~ 5.0 to ~~about~~ 5.8, or ~~about~~ 5.2 to ~~about~~ 5.5.

8. The method of any one of claims 1-7, wherein the lipid nanoparticle solution has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.25, ~~about~~ 4.6 to ~~about~~ 6.0, ~~about~~ 4.8 to ~~about~~ 5.8, ~~about~~ 5.0 to ~~about~~ 5.75, ~~about~~ 5.0 to ~~about~~ 5.5.

9. The method of any one of claims 1-8, wherein the lipid nanoparticle formulation has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.0, ~~about~~ 4.6 to ~~about~~ 5.8, ~~about~~ 4.8 to ~~about~~ 5.6, ~~about~~ 5.0 to ~~about~~ 5.5, or ~~about~~ 5.1 to ~~about~~ 5.4.

10. The method of any one of claims 1-9, wherein the lipid nanoparticle solution or the lipid nanoparticle formulation has a pH that is less than the pKa of the ionizable lipid.

11. The method of any one of claims 1-10, wherein the nucleic acid is a ribonucleic acid.

12. The method of claim 11, wherein the ribonucleic acid is one or more ribonucleic acid selected from a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), a messenger RNA (mRNA), and a long non-coding RNA (lncRNA).

13. The method of claim 12, wherein the nucleic acid is a messenger RNA (mRNA).

14. The method of any one of claims 1-13, wherein the lipid solution and/or the lipid nanoparticle solution further comprises a first organic solvent.

15. The method of any one of claims 1-14, wherein the aqueous buffer solution, nucleic acid solution, and/or the lipid nanoparticle solution comprises a first aqueous buffer.

16. The method of any one of claims 1-15, wherein the aqueous buffer solution, nucleic acid solution, and/or the lipid nanoparticle solution further comprises a second aqueous buffer, a second organic solvent, or both.

17. The method of claim 16, wherein the first aqueous buffer and the second aqueous buffer are at least one buffer selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

18. The method of claim 16, wherein the first organic solvent, the second organic solvent, or both are an alcohol.

19. The method of claim 18, wherein the first organic solvent, the second organic solvent, or both are ethanol.

20. The method of any one of claims 1-19, wherein the lipid solution, the nucleic acid solution, or both further comprise a third organic solvent.

21. The method of claim 20, wherein the third organic solvent is an alcohol.

22. The method of claim 21, wherein the third organic solvent is benzyl alcohol.

23. The method of any one of claims 1-22, further comprising processing the lipid nanoparticle formulation.

24. The method of claim 23, wherein the processing comprises one or more step selected from filtering, pH adjusting, buffer exchanging, dialyzing, concentrating, freezing, lyophilizing, or packing.

25. A method of administering a lipid nanoparticle (LNP) formulation to a patient, the method comprising:

providing an active agent solution having a pH in a range of ~~about~~ 4.5 to ~~about~~ 8.0 comprising a therapeutic and/or prophylactic agent and a lipid nanoparticle solution having a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.5 comprising a lipid nanoparticle, the lipid nanoparticle comprising an ionizable lipid;

forming a lipid nanoparticle formulation comprising the lipid nanoparticle associated with the therapeutic and/or prophylactic agent by mixing the lipid nanoparticle solution and the active agent solution such that the lipid nanoparticle formulation has a pH in a range of ~~about~~ 4.5 to ~~about~~ less than 8.0; and

administering the lipid nanoparticle formulation to the patient after the mixing.

26. The method of claim 25, wherein the lipid nanoparticle of the lipid nanoparticle solution is an empty lipid nanoparticle.

27. The method of claim 25 or 26, wherein the lipid nanoparticle of the lipid nanoparticle solution has an average diameter of ~~about~~ 25 nm to ~~about~~ 125 nm, ~~about~~ 30 nm to ~~about~~ 110 nm, ~~about~~ 35 nm to ~~about~~ 100 nm, ~~about~~ 40 nm to ~~about~~ 90 nm, ~~about~~ 45 nm to ~~about~~ 80 nm, or ~~about~~ 50 nm to ~~about~~ 70 nm.

28. The method of any one of claims 25-27, wherein the active agent solution has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.5, ~~about~~ 4.8 to ~~about~~ 6.25, ~~about~~ 4.8 to ~~about~~ 6.0, ~~about~~ 5.0 to ~~about~~ 5.8, or ~~about~~ 5.2 to ~~about~~ 5.5.

29. The method of any one of claims 25-28, wherein the lipid nanoparticle solution has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.25, ~~about~~ 4.6 to ~~about~~ 6.0, ~~about~~ 4.8 to ~~about~~ 5.8, ~~about~~ 5.0 to ~~about~~ 5.75, ~~about~~ 5.0 to ~~about~~ 5.5.

30. The method of any one of claims 25-29, wherein the lipid nanoparticle formulation has a pH in a range of ~~about~~ 4.5 to ~~about~~ 6.0, ~~about~~ 4.6 to ~~about~~ 5.8, ~~about~~ 4.8 to ~~about~~ 5.6, ~~about~~ 5.0 to ~~about~~ 5.5, or ~~about~~ 5.1 to ~~about~~ 5.4.

31. The method of any one of claims 25-30, wherein the lipid nanoparticle solution has a pH that is less than the pKa of the ionizable lipid.

32. The method of any one of claims 25-31, wherein the lipid nanoparticle formulation has a pH that is less than the pKa of the ionizable lipid.

33. The method of any one of claims 25-32, wherein the pH of the active agent solution and the lipid nanoparticle solution are ~~about~~ the same.

34. The method of any one of claims 25-33, wherein the pH of the active agent solution and the lipid nanoparticle formulation are ~~about~~ the same.

35. The method of any one of claims, wherein a residence time between the mixing and the adding is in a range of ~~about~~ 1.0 milliseconds to ~~about~~ 60 minutes, ~~about~~ 2.0 milliseconds to ~~about~~ 30 minutes, ~~about~~ 3.0 milliseconds to ~~about~~ 15 minutes, ~~about~~ 4.0 milliseconds to ~~about~~ 10 minutes, ~~about~~ 5.0 milliseconds to ~~about~~ 5 minutes ~~about~~ 10.0 milliseconds to ~~about~~ 2 minutes, ~~about~~ 100.0 milliseconds to ~~about~~ 1.0 minute, ~~about~~ 1000 milliseconds to ~~about~~ 1.0 minute.

36. The method of any one of the preceding claims 25-35, wherein the administering is performed less than ~~about~~ 120 minutes after the mixing, less than ~~about~~ 100 minutes after the mixing, less than ~~about~~ 90 minutes after the mixing, less than ~~about~~ 80 minutes after the mixing,

less than ~~about~~ 70 minutes after the mixing, less than ~~about~~ 60 minutes after the mixing, less than ~~about~~ 50 minutes after the mixing, less than ~~about~~ 40 minutes after the mixing, less than ~~about~~ 30 minutes after the mixing, less than ~~about~~ 20 minutes after the mixing, less than ~~about~~ 15 minutes after the mixing, less than ~~about~~ 10 minutes after the mixing.

37. The method of any one of claims 25-36, wherein the therapeutic and/or prophylactic agent is a nucleic acid.

38. The method of claim 37, wherein the nucleic acid solution comprises ~~about~~ 0.01 to ~~about~~ 1.0 mg/mL of the nucleic acid, ~~about~~ 0.05 to ~~about~~ 0.5 mg/mL of the nucleic acid, or ~~about~~ 0.1 to ~~about~~ 0.25 mg/mL of the nucleic acid.

39. The method of claim 37, wherein the nucleic acid is a ribonucleic acid.

40. The method of claim 39, wherein the ribonucleic acid is at least one ribonucleic acid selected from the group consisting of a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), a messenger RNA (mRNA), and a long non-coding RNA (lncRNA).

41. The method of claim 39, wherein the nucleic acid is a messenger RNA (mRNA).

42. The method of any one of claims 25-41, further comprising processing the lipid nanoparticle formulation.

43. The method of claim 42, wherein the processing comprises at least one step selected from the group consisting of filtering, pH adjusting, buffer exchanging, dialyzing, concentrating, freezing, lyophilizing, or packing.

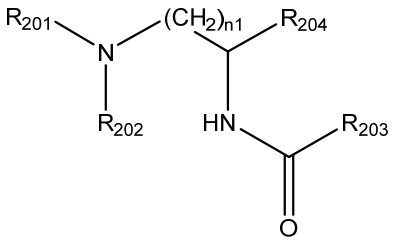
44. The method of claim 43, wherein the filtering is a tangential flow filtration.

45. The method of any one of claims 25-44, wherein the mixing comprises turbulent mixing and/or microfluidic mixing.

46. The method of any one of claims 1-45, wherein the lipid solution, the lipid nanoparticle (LNP) solution, and/or the lipid nanoparticle (LNP) formulation further comprises an encapsulation agent.

47. The method of claim 46, wherein the encapsulation agent is

i) a compound of Formula (EA-I):



(EA-I),

or a salt thereof, wherein

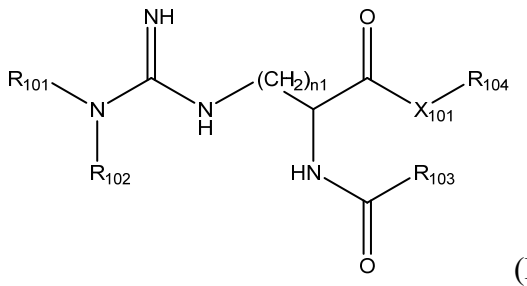
$R_{201}$ and $R_{202}$ are each independently selected from the group consisting of H, $C_1$-$C_6$ alkyl, $C_2$-$C_6$ alkenyl, and $(C{=}NH)N(R_{101})_2$ wherein each $R_{101}$ is independently selected from the group consisting of H, $C_1$-$C_6$ alkyl, and $C_2$-$C_6$ alkenyl;

$R_{203}$ is selected from the group consisting of $C_1$-$C_{20}$ alkyl and $C_2$-$C_{20}$ alkenyl;

$R_{204}$ is selected from the group consisting of H, $C_1$-$C_{20}$ alkyl, $C_2$-$C_{20}$ alkenyl, $C(O)(OC_1$-$C_{20}$ alkyl), $C(O)(OC_2$-$C_{20}$ alkenyl), $C(O)(NHC_1$-$C_{20}$ alkyl), and $C(O)(NHC_2$-$C_{20}$ alkenyl);

n1 is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

ii) a compound of Formula (EA-II):



(EA-II),

or a salt thereof, wherein

$X_{101}$ is a bond, NH, or O;

$R_{101}$ and $R_{102}$ are each independently selected from the group consisting of H, $C_1$-$C_6$ alkyl, and $C_2$-$C_6$ alkenyl;

$R_{103}$ and $R_{104}$ are each independently selected from the group consisting of $C_1$-$C_{20}$ alkyl and $C_2$-$C_{20}$ alkenyl; and

n1 is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; or

iii) ethyl lauroyl arginate or a salt thereof.

48. The method of any one of claims 1-47, wherein the wt/wt ratio of the lipid nanoparticle (LNP) formulation to the nucleic acid is in a range of from ~~about~~ 5:1 to ~~about~~ 60:1.

49. The method of any one of claims 1-48, wherein the lipid nanoparticle or lipid nanoparticle formulation further comprises a phospholipid, a PEG lipid, a structural lipid, or any combination thereof.

50. The method of claim 49, wherein the lipid nanoparticle and/or lipid nanoparticle formulation comprises

~~about~~ 40-60 mol% ionizable lipid;

~~about~~ 5-15 mol% phospholipid;

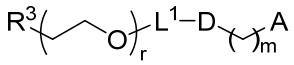
~~about~~ 35-45 mol% structural lipid; or

~~about~~ 0.01-2.0 mol% PEG lipid.

51. The method of claim 49 or 50, wherein the PEG lipid is

i) selected from a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, and a PEG-modified dialkylglycerol;

ii) a compound of Formula (PL-I):



(PL-I),

or salts thereof, wherein:

$R^3$ is $-OR^O$;

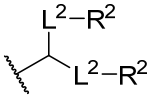
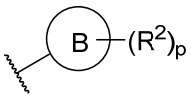
$R^O$ is hydrogen, optionally substituted alkyl, or an oxygen protecting group;

r is an integer between 1 and 100, inclusive;

$L^1$ is optionally substituted $C_{1-10}$ alkylene, wherein at least one methylene of the optionally substituted $C_{1-10}$ alkylene is independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, O, $N(R^N)$, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, or $NR^NC(O)N(R^N)$;

D is a moiety obtained by click chemistry or a moiety cleavable under physiological conditions;

m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

A is of the formula:  or ;

each instance of of $L^2$ is independently a bond or optionally substituted $C_{1-6}$ alkylene, wherein one methylene unit of the optionally substituted $C_{1-6}$ alkylene is optionally replaced with O, $N(R^N)$, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, or $NR^NC(O)N(R^N)$;

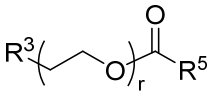
each instance of $R^2$ is independently optionally substituted $C_{1-30}$ alkyl, optionally substituted $C_{1-30}$ alkenyl, or optionally substituted $C_{1-30}$ alkynyl; optionally wherein one or more methylene units of $R^2$ are independently replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, $NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), -OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, $NR^NC(S)N(R^N)$, S(O) , OS(O), S(O)O, -OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, $S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, $N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or -$N(R^N)S(O)_2O$;

each instance of $R^N$ is independently hydrogen, optionally substituted alkyl, or a nitrogen protecting group;

Ring B is optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

p is 1 or 2;

iii) a compound of Formula (PL-II):



(PL-II),

or a salt thereof, wherein:

$R^3$ is–$OR^O$;

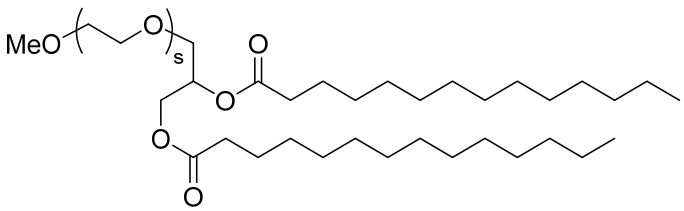
$R^O$ is hydrogen, optionally substituted alkyl or an oxygen protecting group;

r is an integer between 1 and 100;

$R^5$ is optionally substituted $C_{10-40}$ alkyl, optionally substituted $C_{10-40}$ alkenyl, or optionally substituted $C_{10-40}$ alkynyl; and optionally one or more methylene groups of $R^5$ are replaced with optionally substituted carbocyclylene, optionally substituted heterocyclylene, optionally substituted arylene, optionally substituted heteroarylene, $N(R^N)$, O, S, C(O), $C(O)N(R^N)$, -$NR^NC(O)$, $NR^NC(O)N(R^N)$, C(O)O, OC(O), OC(O)O, $OC(O)N(R^N)$, $NR^NC(O)O$, C(O)S, SC(O), $C(=NR^N)$, $C(=NR^N)N(R^N)$, $NR^NC(=NR^N)$, $NR^NC(=NR^N)N(R^N)$, C(S), $C(S)N(R^N)$, $NR^NC(S)$, -$NR^NC(S)N(R^N)$, S(O), OS(O), S(O)O, OS(O)O, $OS(O)_2$, $S(O)_2O$, $OS(O)_2O$, $N(R^N)S(O)$, -$S(O)N(R^N)$, $N(R^N)S(O)N(R^N)$, $OS(O)N(R^N)$, $N(R^N)S(O)O$, $S(O)_2$, $N(R^N)S(O)_2$, $S(O)_2N(R^N)$, -$N(R^N)S(O)_2N(R^N)$, $OS(O)_2N(R^N)$, or $N(R^N)S(O)_2O$; and

each instance of $R^N$ is independently hydrogen, optionally substituted alkyl, or a nitrogen protecting group; or

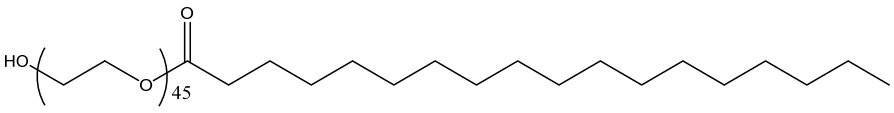
iv) a compound of Formula (PL-III):



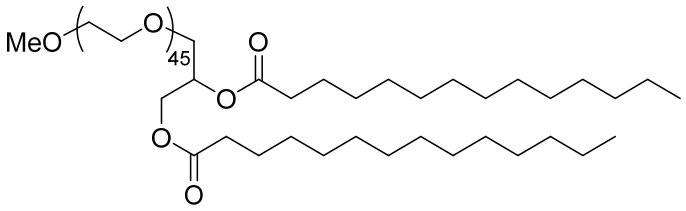
(PL-III),

or a salt thereof, wherein s is an integer between 1 and 100.

52. The method of claim 51, wherein the PEG lipid is




(PEG-1) or




(PEG-2).

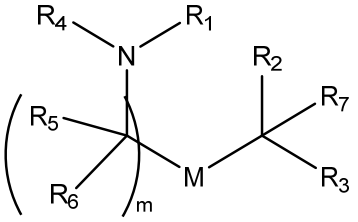
53. The method of any one of claims 49-52, wherein the structural lipid is selected from cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and alpha-tocopherol.

54. The method of any one of claims 49-53, wherein the phospholipid is selected from 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-*sn*-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-*sn*-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine,1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin.

55. The method of any one of claims 49-54, wherein the ionizable lipid

i) comprises an ionizable amino lipid;

ii) is a compound of Formula (IL-1):




(IL-1),

or a salt thereof, wherein:

$R_1$ is selected from the group consisting of $C_{5-30}$ alkyl, $C_{5-20}$ alkenyl, -R*YR", -YR", and -R"M'R';

$R_2$ and $R_3$ are independently selected from the group consisting of H, $C_{1-14}$ alkyl, $C_{2-14}$ alkenyl, -R*YR", -YR", and -R*OR", or $R_2$ and $R_3$, together with the atom to which they are attached, form a heterocycle or carbocycle;

$R_4$ is selected from the group consisting of hydrogen, a $C_{3-6}$ carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted $C_{1-6}$ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $N(R)S(O)_2R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;

each $R_5$ is independently selected from the group consisting of $C_{1-3}$ alkyl, $C_{2-3}$ alkenyl, and H;

each $R_6$ is independently selected from the group consisting of $C_{1-3}$ alkyl, $C_{2-3}$ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -OC(O)-M"-C(O)O-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$,-S-S-, an aryl group, and a heteroaryl group, in which M" is a bond, $C_{1-13}$ alkyl or $C_{2-13}$ alkenyl;

$R_7$ is selected from the group consisting of $C_{1-3}$ alkyl, $C_{2-3}$ alkenyl, and H;

$R_8$ is selected from the group consisting of $C_{3-6}$ carbocycle and heterocycle;

$R_9$ is selected from the group consisting of H, CN, $NO_2$, $C_{1-6}$ alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, $C_{2-6}$ alkenyl, $C_{3-6}$ carbocycle and heterocycle;

each R is independently selected from the group consisting of $C_{1-3}$ alkyl, $C_{2-3}$ alkenyl, and H;

each R' is independently selected from the group consisting of $C_{1-18}$ alkyl, $C_{2-18}$ alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of $C_{3-15}$ alkyl and $C_{3-15}$ alkenyl;

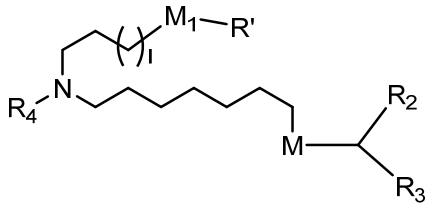
each R* is independently selected from the group consisting of $C_{1-12}$ alkyl and $C_{2-12}$ alkenyl;

each Y is independently a $C_{3-6}$ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13; and wherein when $R_4$ is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2;

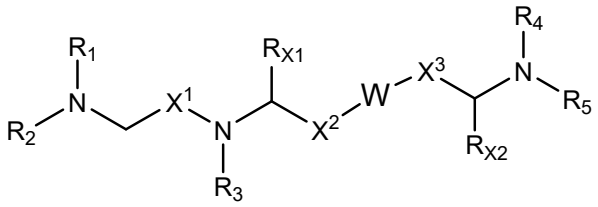
iii) is a compound of Formula (IL-II):



(IL-II)

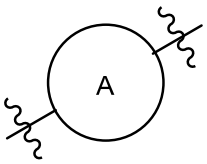
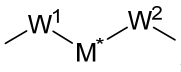
or a salt thereof, wherein l is selected from 1, 2, 3, 4 and 5; M1 is a bond or M'; $R_4$ is hydrogen, unsubstituted $C_{1-3}$ alkyl, or $-(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is –OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -OC(O)-M"- C(O)O-, -C(O)N(R')-, $-P(O)(OR')O-$, -S-S-, an aryl group, and a heteroaryl group,; and $R_2$ and $R_3$ are independently selected from the group consisting of H, $C_{1-14}$ alkyl, and $C_{2-14}$ alkenyl; or

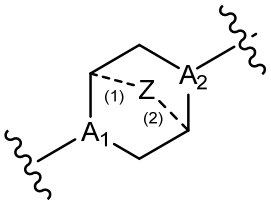
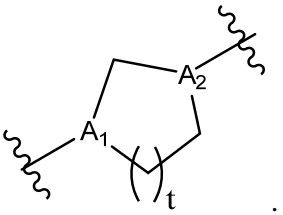
iv) is a compound of formula (IL-III):



(IL-III),

or salts or isomers thereof, wherein,

W is  or ,

ring A is  or ;

t is 1 or 2;

$A_1$ and $A_2$ are each independently selected from CH or N;

Z is $CH_2$ or absent wherein when Z is $CH_2$, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

$R_1$, $R_2$, $R_3$, $R_4$, and $R_5$ are independently selected from the group consisting of $C_{5-20}$ alkyl, $C_{5-20}$ alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

$R_{X1}$ and $R_{X2}$ are each independently H or $C_{1-3}$ alkyl;

each M is independently selected from the group consisting of -C(O)O-, -OC(O)-, -OC(O)O-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -C(O)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is $C_1$-$C_6$ alkyl,

$W^1$ and $W^2$ are each independently selected from the group consisting of -O- and $-N(R_6)-$;

each $R_6$ is independently selected from the group consisting of H and $C_{1-5}$ alkyl;

$X^1$, $X^2$, and $X^3$ are independently selected from the group consisting of a bond, -$CH_2$-, -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(O)O-, -OC(O)-, -$(CH_2)_n$-C(O)-, -C(O)-$(CH_2)_n$-, -$(CH_2)_n$-C(O)O-, -OC(O)-$(CH_2)_n$-, -$(CH_2)_n$-OC(O)-, -C(O)O-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a $C_{3-6}$ carbocycle;

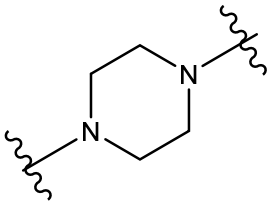
each R* is independently selected from the group consisting of $C_{1-12}$ alkyl and $C_{2-12}$ alkenyl;

each R is independently selected from the group consisting of $C_{1-3}$ alkyl and a $C_{3-6}$ carbocycle;

each R' is independently selected from the group consisting of $C_{1-12}$ alkyl, $C_{2-12}$ alkenyl, and H;

each R" is independently selected from the group consisting of $C_{3-12}$ alkyl, $C_{3-12}$ alkenyl and -R*MR'; and

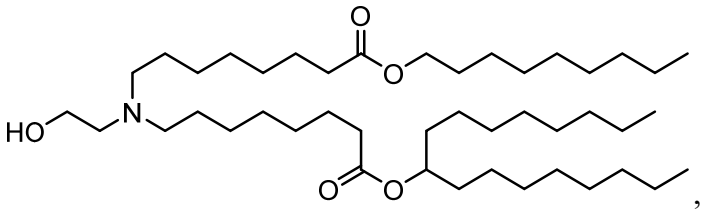
n is an integer from 1-6;

wherein when ring A is , then

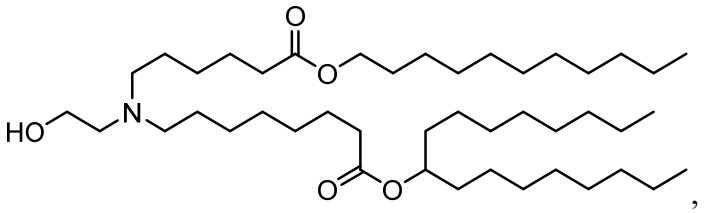
i) at least one of $X^1$, $X^2$, and $X^3$ is not -$CH_2$-; and/or

ii) at least one of $R_1$, $R_2$, $R_3$, $R_4$, and $R_5$ is -R"MR'.

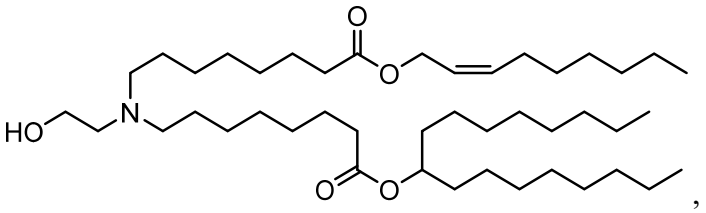
56. The method of claim 55, wherein the ionizable lipid is



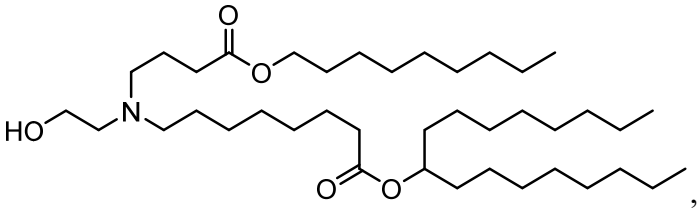
,



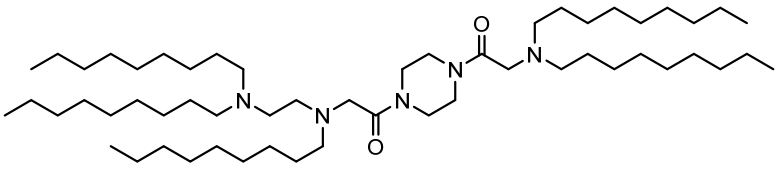
,



,



, or



,

or a salt thereof.

57. The method of claim 55, wherein the ionizable lipid is selected from 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-

1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)).

58. A lipid nanoparticle (LNP) formulation being prepared by the method of any one of claims 1-57.

59. Use of the lipid nanoparticle formulation of any one of claims 1-57 in the manufacture of a medicament for treating or preventing a disease or disorder in a subject.