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, 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 150 nm, less than 125 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 75 nm, less than 70 nm, less than 65 nm, less than 60 nm, less than 55 nm, less than 50 nm, less than 45 nm, less than 40 nm, less than 35 nm, or less than 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 25 nm to 125 nm, 30 nm to 110 nm, 35 nm to 100 nm, 40 nm to 90 nm, 45 nm to 80 nm, or 50 nm to 70 nm.

5. The method of any one of claims 1-4, wherein the aqueous buffer solution has a pH in a range of 4.9 to 5.25.

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

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

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

9. The method of any one of claims 1-8, wherein the lipid nanoparticle formulation has a pH in a range of 4.5 to 6.0, 4.6 to 5.8, 4.8 to 5.6, 5.0 to 5.5, or 5.1 to 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 4.5 to 8.0 comprising a therapeutic and/or prophylactic agent and a lipid nanoparticle solution having a pH in a range of 4.5 to 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 4.5 to 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 25 nm to 125 nm, 30 nm to 110 nm, 35 nm to 100 nm, 40 nm to 90 nm, 45 nm to 80 nm, or 50 nm to 70 nm.

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

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

30. The method of any one of claims 25-29, wherein the lipid nanoparticle formulation has a pH in a range of 4.5 to 6.0, 4.6 to 5.8, 4.8 to 5.6, 5.0 to 5.5, or 5.1 to 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 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 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 1.0 milliseconds to 60 minutes, 2.0 milliseconds to 30 minutes, 3.0 milliseconds to 15 minutes, 4.0 milliseconds to 10 minutes, 5.0 milliseconds to 5 minutes 10.0 milliseconds to 2 minutes, 100.0 milliseconds to 1.0 minute, 1000 milliseconds to 1.0 minute.

36. The method of any one of the preceding claims 25-35, wherein the administering is performed less than 120 minutes after the mixing, less than 100 minutes after the mixing, less than 90 minutes after the mixing, less than 80 minutes after the mixing, less than 70 minutes after the mixing, less than 60 minutes after the mixing, less than 50 minutes after the mixing, less than 40 minutes after the mixing, less than 30 minutes after the mixing, less than 20 minutes after the mixing, less than 15 minutes after the mixing, less than 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 0.01 to 1.0 mg/mL of the nucleic acid, 0.05 to 0.5 mg/mL of the nucleic acid, or 0.1 to 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.

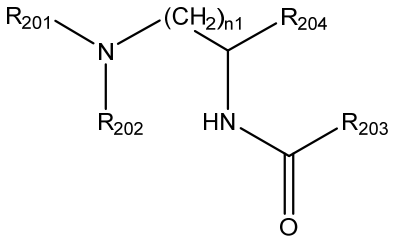
43. The method of claim 42, wherein the processing comprises at least one step selected from the group consisting of filtering, pH adjusting, buffer exchanging, dialyzing, concentrating, freezing, lyophilizing, or packing.

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

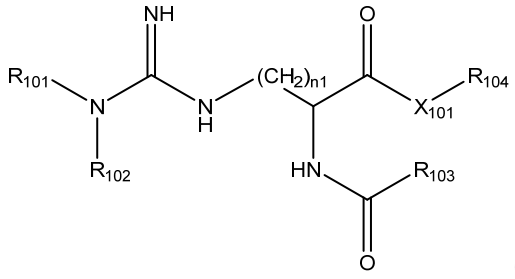
R_{201} and R_{202} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, and $(C=NH)N(R_{101})_2$ wherein each R_{101} is independently selected from the group consisting of H, C_1-C_6 alkyl, and C_2-C_6 alkenyl;

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 5:1 to 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

40-60 mol% ionizable lipid;

5-15 mol% phospholipid;

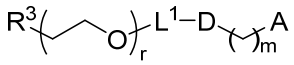
35-45 mol% structural lipid; or

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 $–OR^O$;

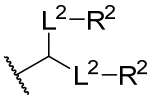
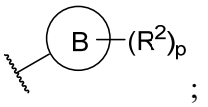
R^O is hydrogen, optionally substituted alkyl, or an oxygen protecting group;

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 $NR^NC(O)N(R^N)$;

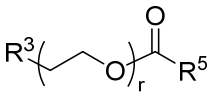
each instance of R^2 is independently optionally substituted C_{1-30} alkyl, optionally substituted C_{1-30} alkenyl, or optionally substituted C_{1-30} alkynyl; optionally wherein one or more methylene units of R^2 are independently replaced with optionally substituted carbocyclylene, optionally substituted 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 $–OR^O$;

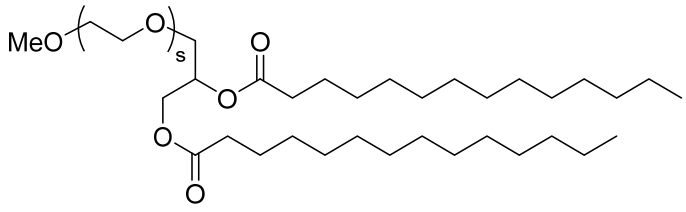
R^O is hydrogen, optionally substituted alkyl or an oxygen protecting group;

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

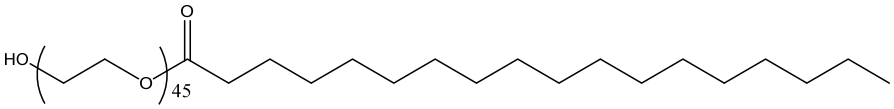
iv) a compound of Formula (PL-III):



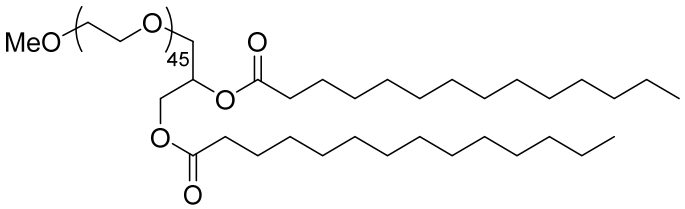
(PL-III),

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

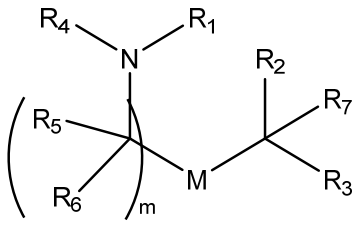


(PEG-2).

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;

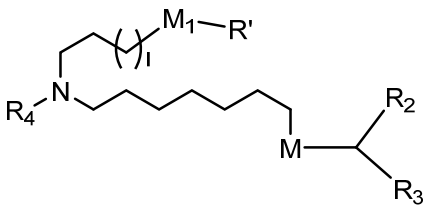
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

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;

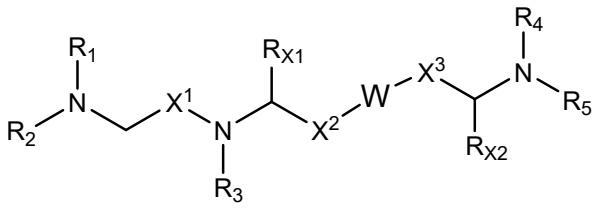
iii) is a compound of Formula (IL-II):



(IL-II)

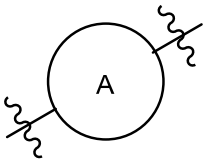
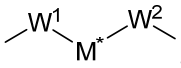
or a salt thereof, wherein l is selected from 1, 2, 3, 4 and 5; M1 is a bond or M'; R_4 is hydrogen, unsubstituted C_{1-3} alkyl, or $–(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is –OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $- NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, 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

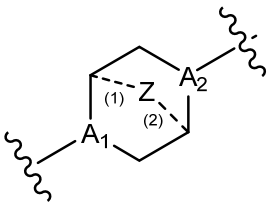
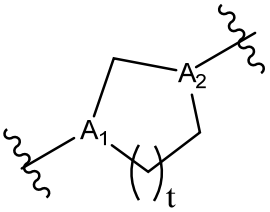
iv) is a compound of formula (IL-III):



(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;

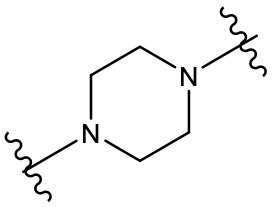
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

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

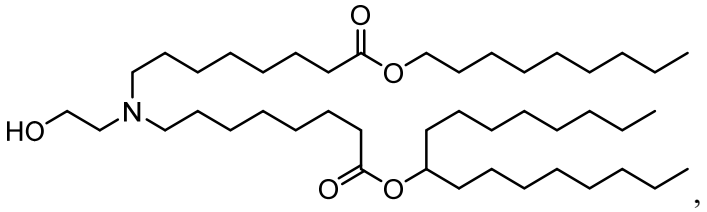
n is an integer from 1-6;

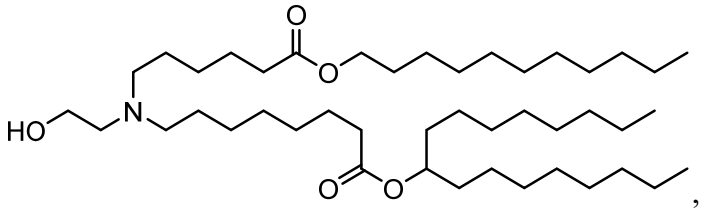
wherein when ring A is , then

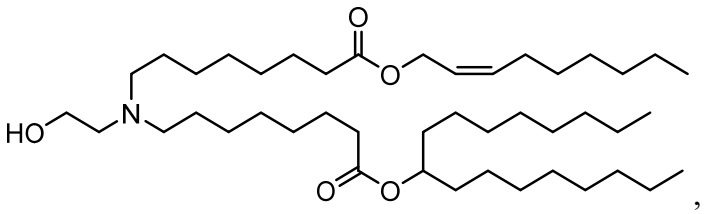
i) at least one of X^1, X^2, and X^3 is not -CH_2-; and/or

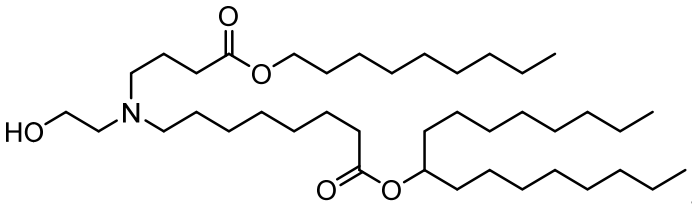
ii) at least one of R_1, R_2, R_3, R_4, and R_5 is -R"MR'.

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

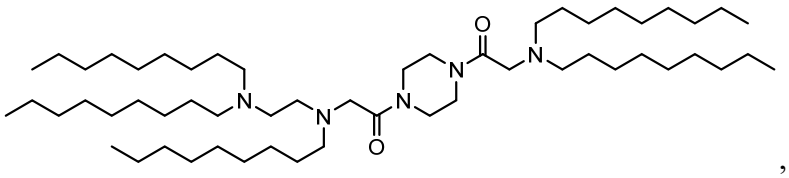
,

,

,



, 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.