CLAIMS

1. A method of preparing an empty-lipid nanoparticle solution (empty-LNP solution) comprising an empty lipid nanoparticle (empty LNP), comprising:

(i) a mixing step, comprising mixing a lipid solution comprising an ionizable lipid, a phospholipid, a PEG lipid and a structural lipid with an aqueous buffer solution comprising a first buffering agent, thereby forming the empty-LNP solution comprising the empty LNP, wherein the empty LNP comprises from about 0.1 mol % to about 0.5 mol % of the PEG lipid,

wherein the empty-LNP solution comprises an acetate buffer and has a pH in the range of about 4.6 to about 6.0.

2. The method of claim 1, further comprising processing the empty-LNP solution.

3. A method of preparing a loaded lipid nanoparticle solution (loaded-LNP solution) comprising a loaded lipid nanoparticle (loaded LNP), comprising:

(i) a mixing step, comprising mixing a lipid solution comprising an ionizable lipid, a phospholipid, a PEG lipid and a structural lipid with an aqueous buffer solution comprising a first buffering agent, thereby forming the empty-LNP solution comprising the empty LNP, wherein the empty LNP comprises from about 0.1 mol % to about 0.5 mol % of the PEG lipid, wherein the empty-LNP solution comprises an acetate buffer and has a pH in the range of about 4.6 to about 6.0; and

(ii) a loading step, comprising mixing a nucleic acid solution comprising a nucleic acid with the empty-LNP solution, thereby forming a loaded-LNP solution comprising a loaded LNP.

4. The method of any one of the preceding claims, further comprising processing the loaded-LNP solution, thereby forming a lipid nanoparticle formulation (LNP formulation).

5. The method of any one of the preceding claims, wherein the step of processing the loaded-LNP solution comprises a first adding step, comprising adding a polyethylene glycol lipid (PEG lipid) to the loaded LNP.

6. The method of any one of the preceding claims, wherein the first adding step comprises adding a polyethylene glycol solution (PEG solution) comprising the PEG lipid to the loaded-LNP solution.

7. The method of any one of the preceding claims, wherein the first adding step comprises adding from about 0.1 mol% to about 3.0 mol% PEG lipid, from about 0.2 mol% to about 2.5 mol% PEG lipid, from about 0.5 mol% to about 2.0 mol% PEG lipid, from about 0.75 mol% to about 1.5 mol% PEG lipid, or from about 1.0 mol% to about 1.25 mol% PEG lipid to the empty LNP or the loaded LNP.

8. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution further comprises pH adjusting.

9. The method of any one of the preceding claims, wherein the pH adjusting comprises adding a second buffering agent.

10. The method of any one of the preceding claims, wherein the second buffering agent comprises a second aqueous buffer solution.

11. The method of any one of the preceding claims, wherein the second aqueous buffer is selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

12. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution further comprises filtering.

13. The method of any one of the preceding claims, wherein the filtering is performed by a tangential flow filtration.

14. The method of any one of the preceding claims, wherein the step of processing the loaded-LNP solution further comprises buffer exchanging.

15. The method of any one of the preceding claims, wherein the buffer exchanging comprises addition of an aqueous buffer solution comprising a third buffering agent.

16. The method of any one of the preceding claims, wherein the third buffering agent comprises a third aqueous buffer solution.

17. The method of any one of the preceding claims, wherein the third aqueous buffer solution is selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

18. The method of any one of the preceding claims, wherein the third aqueous buffer solution has a pH in a range of about 6.5 to about 8.5, about 7.0 to about 8.0, about 7.2 to about 7.8, or about 7.4 to about 7.6.

19. The method of any one of the preceding claims, wherein the third aqueous buffer solution has a pH of about 7.5.

20. The method of any one of the preceding claims, wherein the first adding step is performed prior to the buffer exchanging.

21. The method of any one of the preceding claims, wherein the first adding step is performed after the buffer exchanging.

22. The method of any one of the preceding claims, wherein the step of processing the loaded-LNP solution comprises a second adding step, comprising adding a polyethylene glycol lipid (PEG lipid) to the loaded LNP.

23. The method of any one of the preceding claims, wherein the second adding step is performed prior to the buffer exchanging.

24. The method of any one of the preceding claims, wherein the second adding step is performed after the buffer exchanging.

25. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution further comprises diluting the empty-LNP solution.

26. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution or loaded-LNP solution further comprises freezing the empty-LNP solution or loaded-LNP solution.

27. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution or loaded-LNP solution further comprises lyophilizing the empty-LNP solution or loaded-LNP solution.

28. The method of any one of the preceding claims, wherein the step of processing the empty-LNP solution or loaded-LNP solution further comprises storing the empty-LNP solution or loaded-LNP solution.

29. The method of any one of the preceding claims, wherein the mixing step is performed with a T-junction, confined impinging jets, microfluidic mixer, or vortex mixer.

30. The method of any one of the preceding claims, wherein the loading step is performed with a T-junction, confined impinging jets, microfluidic mixer, or vortex mixer.

31. The method of any one of the preceding claims, wherein the aqueous buffer solution has a pH in a range of from about 4.5 to about 6.5, from about 4.6 to about 6.0, from about 4.7 to about 5.75, from about 4.8 to about 5.5, or from about 4.9 to about 5.25.

32. The method of any one of the preceding claims, wherein the aqueous buffer solution has a pH of about 5.0.

33. The method of any one of the preceding claims, wherein the empty-LNP solution has a pH in a range of from about 4.8 to about 5.8, from about 5.0 to about 5.75, or from about 5.0 to about 5.5.

34. The method of any one of the preceding claims, wherein the nucleic acid solution has a pH in a range of from about 4.5 to about 6.5, from about 4.8 to about 6.25, from about 4.8 to about 6.0, from about 5.0 to about 5.8, or from about 5.2 to about 5.5.

35. The method of any one of the preceding claims, wherein the pH of the nucleic acid solution, the empty-LNP solution, and the LNP formulation are in a range of from about 5.0 to about 6.0, from about 5.1 to about 5.75, or from about 5.2 to about 5.5.

36. The method of any one of the preceding claims, wherein the loaded-LNP solution has a pH in a range of from about 4.5 to about 6.0, from about 4.6 to about 5.8, from about 4.8 to about 5.6, from about 5.0 to about 5.5, or from about 5.1 to about 5.4.

37. The method of any one of the preceding claims, wherein the lipid solution further comprises a first organic solvent.

38. The method of any one of the preceding claims, wherein the empty-LNP solution or loaded-LNP solution further comprises a first organic solvent.

39. The method of any one of the preceding claims, wherein the first organic solvent is an alcohol.

40. The method of any one of the preceding claims, wherein the first organic solvent is ethanol.

41. The method of any one of the preceding claims, wherein the first buffering agent comprises a first aqueous buffer solution.

42. The method of any one of the preceding claims, wherein the first aqueous buffer is selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

43. The method of any one of the preceding claims, wherein the first aqueous buffer solution comprises greater than about 1 mM citrate, acetate, phosphate or tris, greater than about 2 mM citrate, acetate, phosphate or tris, greater than about 5 mM citrate, acetate, phosphate or tris, greater than about 10 mM citrate, acetate, phosphate or tris, greater than about 15 mM citrate, acetate, phosphate or tris, greater than about 20 mM citrate, acetate, phosphate or tris, greater than about 25 mM citrate, acetate, phosphate or tris, or greater than about 30 mM citrate, acetate, phosphate or tris.

44. The method of any one of the preceding claims, wherein the first aqueous buffer solution comprises about 1 mM to about 30 mM citrate, acetate, phosphate or tris, about 2 mM to about 20 mM citrate, acetate, phosphate or tris, about 3 mM to about 10 mM citrate, acetate, phosphate or tris, about 4 mM to about 8 mM citrate, acetate, phosphate or tris, or about 5 mM to about 6 mM citrate, acetate, phosphate or tris.

45. The method of any one of the preceding claims, wherein the first aqueous buffer solution comprises about 5 mM citrate, acetate, phosphate or tris.

46. The method of any one of the preceding claims, wherein the first aqueous buffer solution comprises about 5 mM acetate, wherein the aqueous buffer solution has a pH of about 5.0.

47. The method of any one of the preceding claims, wherein the empty-LNP solution or loaded-LNP solution further comprises a tonicity agent.

48. The method of any one of the preceding claims, wherein the tonicity agent is a sugar.

49. The method of any one of the preceding claims, wherein the sugar is sucrose.

50. The method of any one of the preceding claims, wherein the empty-LNP solution or loaded-LNP solution comprises from about 0.01 g/mL to about 1.0 g/mL, from about 0.05 g/mL to about 0.5 g/mL, from about 0.1 g/mL to about 0.4 g/mL, from about 0.15 g/mL to about 0.3 g/mL, or from about 0.2 g/mL to about 0.25 g/mL tonicity agent.

51. The method of any one of the preceding claims, wherein the empty-LNP solution or loaded-LNP solution further comprises from about 0.2 g/mL to about 0.25 g/mL tonicity agent.

52. The method of any one of the preceding claims, wherein the empty-LNP solution or loaded-LNP solution further comprises about 0.2 g/mL sucrose.

53. The method of any one of the preceding claims, 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.

54. The method of any one of the preceding claims, wherein the nucleic acid solution comprises a buffer selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

55. The method of any one of the preceding claims, wherein the nucleic acid solution comprises an acetate buffer.

56. The method of any one of the preceding claims, wherein the nucleic acid solution comprises from about 1 mM to about 200 mM acetate buffer, from about 2 mM to about 180 mM acetate buffer, from about 3 mM to about 160 mM acetate buffer, from about 4 mM to about 150 mM acetate buffer, from about 4 mM to about 140 mM acetate buffer, from about 5 mM to about 130 mM acetate buffer, from about 6 mM to about 120 mM acetate buffer, from about 7 mM to about 110 mM acetate buffer, from about 8 mM to about 100 mM acetate buffer, from about 9 mM to about 90 mM acetate buffer, from about 10 mM to about 80 mM acetate buffer, from about 15 mM to about 70 mM acetate buffer, from about 20 mM to about 60 mM acetate buffer, from about 25 mM to about 50 mM acetate buffer, or from about 30 mM to about 40 mM acetate buffer.

57. The method of any one of the preceding claims, wherein the nucleic acid solution and the empty-LNP solution are mixed at a volumetric flow ratio of from about 5:1 to about 7:1, from about 4:1 to about 6:1, from about 3:1 to about 5:1, or from about 2:1 to about 4:1 during the loading step.

58. The method of any one of the preceding claims, wherein the loaded-LNP solution comprises an acetate buffer.

59. The method of any one of the preceding claims, wherein the lipid solution, the empty LNP, the empty-LNP solution, the loaded LNP, the loaded-LNP solution, and/or the LNP formulation further comprises an encapsulation agent.

60. The method of any one of the preceding claims, wherein the lipid solution, the empty-LNP solution, the loaded LNP, the loaded-LNP solution, and/or the LNP formulation further comprises a phospholipid, a PEG lipid, a structural lipid, or any combination thereof.

61. The method of any one of the preceding claims, wherein the empty LNP comprises
about 30-60 mol% ionizable lipid;
about 0-30 mol% phospholipid;
about 15-50 mol% structural lipid; and
about 0.1-0.5 mol% PEG lipid.

62. The method of any one of the preceding claims, wherein the PEG lipid is selected from the group consisting of 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.

63. The method of any one of the preceding claims, wherein the structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and derivatives thereof.

64. The method of any one of the preceding claims, wherein the phospholipid is selected from the group consisting of 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), sphingomyelin, and derivatives thereof.

65. The method of any one of the preceding claims, wherein the ionizable lipid comprises an ionizable amino lipid.

66. The method of any one of the preceding claims, wherein the nucleic acid is a ribonucleic acid.

67. The method of any one of the preceding claims, 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).

68. The method of any one of the preceding claims, wherein the nucleic acid is a messenger RNA (mRNA).

69. The method of any one of the preceding claims, wherein the mRNA includes at least one motif selected from the group consisting of a stem loop, a chain terminating nucleoside, a polyA sequence, a polyadenylation signal, and a 5' cap structure.

70. The method of any one of the preceding claims, wherein the mRNA is at least 30 nucleotides in length.

71. The method of any one of the preceding claims, wherein the mRNA is at least 300 nucleotides in length.

72. The method of any one of the preceding claims, wherein the LNP formulation has a N:P ratio from about 1.1:1 to about 30.1.

73. The method of any one of the preceding claims, wherein the LNP formulation has a N:P ratio from about 2:1 to about 20:1.

74. The method of any one of the preceding claims, wherein the LNP formulation has a N:P ratio from about 2:1 to about 10:1 or about 2:1 to about 5:1.

75. The method of any one of the preceding claims, wherein the LNP formulation comprises from about 0.01 to about 500 mg/mL of the nucleic acid, from about 0.1 to about 100 mg/mL, from about 0.25 to about 50 mg/mL, from about 0.5 to about 10 mg/mL, or from about 1.0 to about 10 mg/mL of the nucleic acid.

76. An empty LNP comprising from about 0.1 mol % to about 0.5 mol % of a PEG lipid.

77. An empty LNP prepared by the method of any one of the preceding claims.

78. An empty-LNP solution prepared by the method of any one of the preceding claims.

79. An empty-LNP solution comprising an empty LNP, wherein the empty LNP comprises from about 0.1 mol % to about 0.5 mol % of a PEG lipid.

80. A loaded LNP prepared by the method of any one of the preceding claims.

81. A loaded-LNP solution prepared by the method of any one of the preceding claims.

82. A LNP formulation prepared by the method of any one of the preceding claims.

83. A method of treating or preventing a disease or disorder, the method comprising administering to a subject in need thereof the loaded LNP of any one of the preceding claims.

84. A method of treating or preventing a disease or disorder, the method comprising administering to a subject in need thereof the loaded-LNP solution of any one of the preceding claims.

85. A method of treating or preventing a disease or disorder, the method comprising administering to a subject in need thereof the LNP formulation of any one of the preceding claims.

86. The method of any one of the preceding claims, wherein the administering is performed parenterally.

87. The method of any one of the preceding claims, wherein the administering is performed intramuscularly, intradermally, subcutaneously, and/or intravenously.

88. The loaded LNP of any one of the preceding claims for use in treating or preventing a disease or disorder in a subject.

89. The loaded-LNP solution any one of the preceding claims for use in treating or preventing a disease or disorder in a subject.

90. The LNP formulation of any one of the preceding claims for use in treating or preventing a disease or disorder in a subject.

91. Use of the loaded LNP of any one of the preceding claims in the manufacture of a medicament for treating or preventing a disease or disorder.

92. Use of the loaded-LNP solution of any one of the preceding claims in the manufacture of a medicament for treating or preventing a disease or disorder.

93. Use of the LNP formulation of any one of the preceding claims in the manufacture of a medicament for treating or preventing a disease or disorder.

94. A pharmaceutical kit, comprising the empty LNP, empty-LNP solution, loaded LNP, loaded-LNP solution, or LNP formulation of any one of claims 77-83.

95. An empty LNP comprising from about 0.1 mol % to about 1.25 mol % of a PEG lipid.

96. An empty LNP comprising from about 0.1 mol % to about 0.5 mol % of a PEG lipid.

97. The empty LNP of any one of the preceding claims, further comprising an ionizable lipid.

98. The empty LNP of any one of the preceding claims, further comprising a phospholipid and a structural lipid.

99. An empty LNP comprising about 30-60 mol% ionizable lipid; about 0-30 mol% phospholipid; about 15-50 mol% structural lipid; and about 0.1-10 mol% PEG lipid.

100. An empty-LNP solution comprising the empty LNP of any one of the preceding claims.

101. The empty-LNP solution of any one of the preceding claims, further comprising an acetate buffer.

102. The empty-LNP solution of any one of the preceding claims, further comprising a tonicity agent.

103. The empty-LNP solution of any one of the preceding claims, wherein the tonicity agent is sucrose.

104. An empty-LNP solution comprising:
(i) an empty LNP comprising from about 0.1 mol % to about 1.25 mol % of a PEG lipid; and
(ii) an acetate buffer.

105. An empty-LNP solution comprising:
(i) an empty LNP comprising from about 0.1 mol % to about 1.25 mol % of a PEG lipid;
(ii) an acetate buffer; and
(iii) sucrose.

106. An empty-LNP solution comprising:
(i) an empty LNP comprising from about 0.1 mol % to about 0.5 mol % of a PEG lipid; and
(ii) an acetate buffer.

107. An empty-LNP solution comprising:
(i) an empty LNP comprising from about 0.1 mol % to about 0.5 mol % of a PEG lipid;
(ii) an acetate buffer; and
(iii) sucrose

108. The empty-LNP solution of any one of the preceding claims, having a pH value of from about 4.5 to about 6.25, from about 4.6 to about 6.0, from about 4.8 to about 5.8, from about 5.0 to about 5.75, or from about 5.0 to about 5.5.

109. The empty-LNP solution of any one of the preceding claims, comprising about 5 mM acetate buffer, wherein the acetate buffer has a pH of about 5.0.

110. The empty-LNP solution of any one of the preceding claims, comprising about 0.2 g/mL sucrose.

111. The empty-LNP solution of any one of the preceding claims, wherein the empty LNP comprises from about 30 mol% to about 60 mol% of the ionizable lipid, from about 0 mol% to about 30 mol% of the phospholipid, from about 15 mol% to about 50 mol% of the structural lipid, and from about 0.1 mol% to about 0.5 mol% of the PEG lipid.

112. The empty-LNP solution of any one of the preceding claims, wherein the empty LNP comprises from about 40 mol% to about 60 mol% of the ionizable lipid, from about 5 mol% to about 20 mol% of the phospholipid, from about 30 mol% to about 50 mol% of the structural lipid, and from about 0.1 mol % to about 1.25 mol % of the PEG lipid.

113. The empty-LNP solution of any one of the preceding claims, wherein the PEG lipid is present at a concentration of about 0.2 mol% to about 0.7 mol%.

114. The empty-LNP solution of any one of the preceding claims, wherein the PEG lipid is present at a concentration of about 0.5 mol%.

115. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution comprises an acetate buffer having a concentration of from about 2 mM to about 40 mM.

116. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution comprises an acetate buffer having a concentration of from about 2 mM to about 30 mM.

117. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution comprises an acetate buffer having a concentration of from about 2 mM to about 20 mM.

118. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution comprises an acetate buffer having a concentration of from about 2 mM to about 10 mM.

119. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution comprises an acetate buffer having a concentration of about 5 mM.

120. The empty-LNP solution of any one of the preceding claims, wherein the buffer has a pH of at least 1 unit less than the pKa of the ionizable lipid.

121. The empty-LNP solution of any one of the preceding claims, wherein the buffer has a pH of less than 5.5.

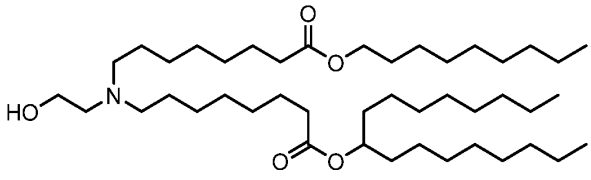
122. The empty-LNP solution of any one of the preceding claims, wherein the buffer has a pH of about 5.0.

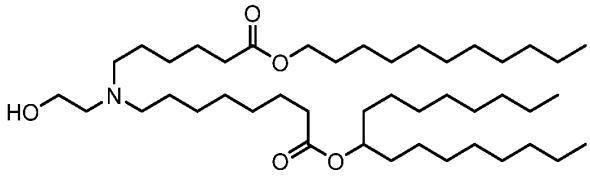
123. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution has a pH of at least 1 unit less than the pKa of the ionizable lipid.

124. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution has a pH of less than 5.5.

125. The empty-LNP solution of any one of the preceding claims, wherein the empty-LNP solution has a pH of about 5.0.

126. The empty-LNP solution of any one of the preceding claims, wherein the LNPs comprise about 45 mol % to about 50 mol % ionizable lipid.

127. The empty-LNP solution of any one of the preceding claims, wherein the ionizable lipid is , or a salt thereof.

128. The empty-LNP solution of any one of the preceding claims, wherein the ionizable lipid is , or a salt thereof.

129. The empty-LNP solution of any one of the preceding claims, wherein the PEG lipid is PEG_{2k}-DMG.

130. The empty-LNP solution of any one of the preceding claims, wherein the structural lipid is cholesterol.

131. The empty-LNP solution of any one of the preceding claims, wherein the phospholipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).

132. A preparation comprising lipid nanoparticles (LNPs), wherein:

(a) the LNPs comprise

from about 40 mol % to about 50 mol % ionizable lipid,

from about 30 mol % to about 50 mol % structural lipid,

from about 5 mol % to about 20 mol % phospholipid, and

from about 0.1 mol % to about 1.25 mol % of a PEG lipid;

(b) the LNPs are substantially free of a therapeutic or prophylactic agent; and

(c) the preparation comprises an acetate buffer having a concentration of from about 2 mM to about 40 mM.

133. The preparation of any one of the preceding claims, wherein the PEG lipid is present at a concentration of about 0.2 mol% to about 0.7 mol %.

134. The preparation of any one of the preceding claims, wherein the PEG lipid is present at a concentration of about 0.5 mol %.

135. The preparation of any one of the preceding claims, wherein the preparation comprises an acetate buffer having a concentration of from about 2 mM to about 30 mM.

136. The preparation of any one of the preceding claims, wherein the preparation comprises an acetate buffer having a concentration of from about 2 mM to about 20 mM.

137. The preparation of any one of the preceding claims, wherein the preparation comprises an acetate buffer having a concentration of from about 2 mM to about 10 mM.

138. The preparation of any one of the preceding claims, wherein the preparation comprises an acetate buffer having a concentration of about 5 mM.

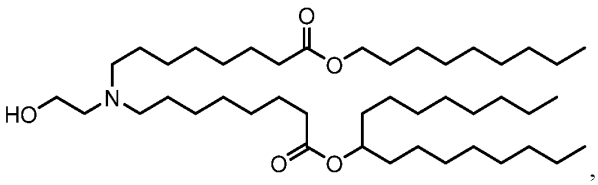
139. The preparation of any one of the preceding claims, wherein the buffer has a pH of at least 1 unit less than the pKa of the ionizable lipid.

140. The preparation of any one of the preceding claims, wherein the buffer has a pH of less than 5.5.

141. The preparation of any one of the preceding claims, wherein the buffer has a pH of about 5.0.

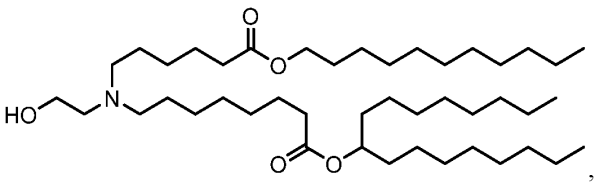
142. The preparation of any one of the preceding claims, wherein the LNPs comprise about 45 mol % to about 50 mol % ionizable lipid.

143. The preparation of any one of the preceding claims, wherein the ionizable lipid is



, or a salt thereof.

144. The preparation of any one of the preceding claims, wherein the ionizable lipid is



, or a salt thereof.

145. The preparation of any one of the preceding claims, wherein the PEG lipid is PEG_{2k}-DMG.

146. The preparation of any one of the preceding claims, wherein the structural lipid is cholesterol.

147. The preparation of any one of the preceding claims, wherein the phospholipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).