CLAIMS

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

i) a nanoprecipitation step, comprising:

i-a) mixing a lipid solution comprising an ionizable lipid, a structural lipid, and a phospholipid, with an aqueous buffer solution comprising a first buffering agent, thereby forming an intermediate empty-lipid nanoparticle solution (intermediate empty-LNP solution) comprising an intermediate empty nanoparticle (intermediate empty LNP);

i-b) holding the intermediate empty-LNP solution for a residence time; and

i-c) adding a diluting solution to the intermediate empty-LNP solution, thereby forming the empty-LNP solution.

2. The method of claim 1, wherein the aqueous buffer solution has a pH value being higher than the pKa value of the ionizable lipid.

3. The method of claim 1 or 2, wherein the aqueous buffer solution has a pH value of 8.0±2.0, 8.0±1.5, 8.0±1.0, 8.0±0.9, 8.0±0.8, 8.0±0.7, 8.0±0.6, 8.0±0.5, 8.0±0.4, 8.0±0.3, 8.0±0.2, or 8.0±0.1.

4. The method of any one of claims 1-3, wherein the aqueous buffer solution comprises phosphate.

5. The method of any one of claims 1-4, wherein the lipid solution further comprises a PEG lipid.

6. The method of any one of claims 1-4, wherein the lipid solution is free of PEG lipid.

7. The method of any one of claims 1-6, wherein the residence time is less than about one second, from about one second to about one minute, or from about one minute to about one hour.

8. The method of any one of claims 1-7, wherein the diluting solution has a pH value being lower than the pKa value of the ionizable lipid.

9. The method of any one of claims 1-8, wherein the diluting solution has a pH value of 5.0±2.0, 5.0±1.5, 5.0±1.0, 5.0±0.9, 5.0±0.8, 5.0±0.7, 5.0±0.6, 5.0±0.5, 5.0±0.4, 5.0±0.3, 5.0±0.2, or 5.0±0.1.

10. The method of any one of claims 1-9, wherein the diluting solution comprises acetate.

11. The method of any one of claims 1-10, wherein the diluting solution is free of PEG lipid.

12. The method of any one of claims 1-11, wherein the diluting solution further comprises a PEG lipid.

13. The method of any one of claims 1-12, further comprising:
i-d) filtering the empty-LNP solution after step i-c).

14. The method of any one of claims 1-13, further comprising ii) processing the empty-LNP solution, thereby forming an empty-LNP formulation.

15. The method of claim 14, wherein processing the empty-LNP solution comprises adding a cryoprotectant.

16. The method of claim 14 or 15, wherein the empty-LNP formulation has a pH value being lower than the pKa value of the ionizable lipid.

17. The method of any one of claims 1-16, further comprising iii) mixing a nucleic acid solution comprising a nucleic acid with the empty-LNP solution or the empty-LNP formulation, thereby forming the loaded-LNP solution comprising a loaded lipid nanoparticle (loaded LNP).

18. The method of claim 17, further comprising adding a pre-loading buffering solution to the empty-LNP solution or empty-LNP formulation prior to step iii).

19. The method of claim 17, wherein the nucleic acid is mRNA.

20. The method of any one of claims 17-19, wherein the loaded-LNP solution has a pH value being lower than the pKa value of the ionizable lipid.

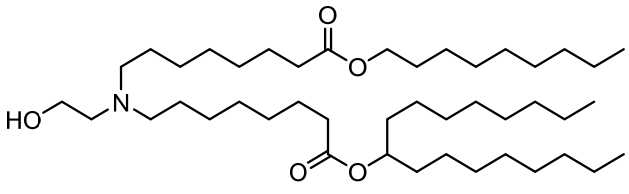
21. The method of any one of claims 1-20, further comprising iv) processing the loaded-LNP solution, thereby forming a loaded-LNP formulation.

22. The method of claim 21, further comprising:

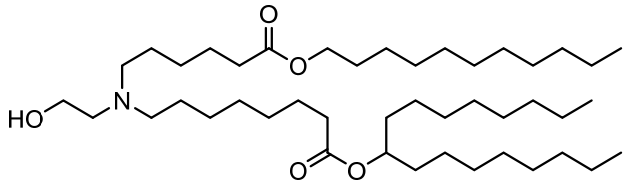
iii-a) holding the loaded-LNP solution for 5 seconds or longer prior to processing the loaded-LNP solution.

23. The method of claim 21 or 22, wherein processing the loaded-LNP solution comprises adding the PEG lipid to the loaded-LNP solution.

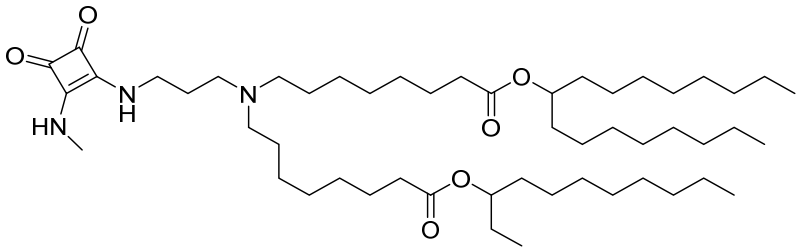
24. The method of any one of claims 1-23, wherein the ionizable lipid is



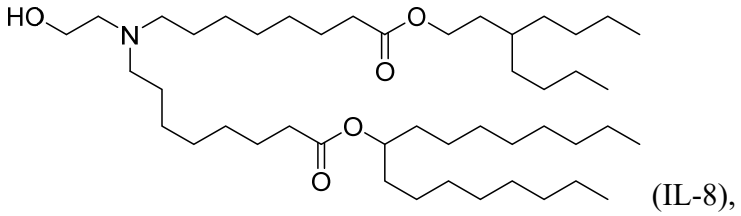
(IL-1),



(IL-2),



(IL-6), or



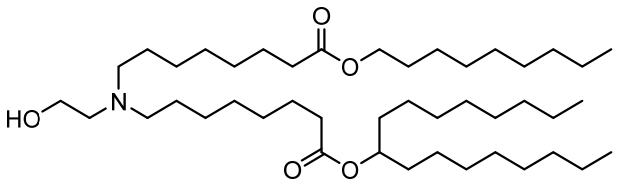
(IL-8),

or a salt thereof.

25. The method of any one of claims 1-24, wherein the structural lipid is cholesterol, fecosterol, sitosterol, ergosterol, campesterol, or stigmasterol.

26. The method of any one of claims 1-25, wherein the phospolipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 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-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-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), or sphingomyelin.

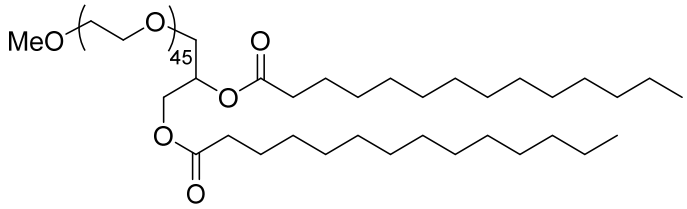
27. The method of any one of claims 1-26, wherein the ionizable lipid is



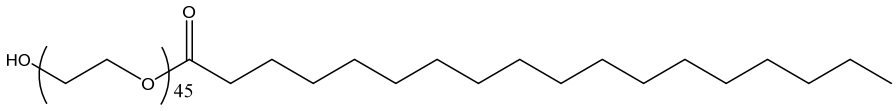
(IL-1),

the structural lipid is cholesterol, and the phospolipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).

28. The method of any one of claims 5-27, wherein the PEG lipid is PEG_{2k}-DMG or PEG-1



(PEG_{2k}-DMG) or



(PEG-1).

29. A population of empty-LNPs prepared by the method of any one of claims 1-28.

30. Use of the loaded-LNP solution or loaded-LNP formulation prepared by the method of any one of claims 17-28 in the manufacture of a medicament for treating or preventing a disease or disorder.