CLAIMS:

1. A lipid composition comprising:

a biologically active agent; and

a lipid component, wherein the lipid component comprises:

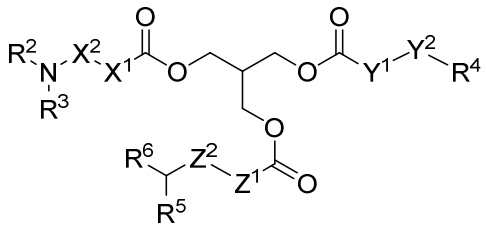
a) an ionizable lipid in an amount from about 25-45 mol % of the lipid component;

b) a neutral lipid in an amount from about 10-30 mol % of the lipid component;

c) a helper lipid in an amount from about 25-65 mol % of the lipid component; and

d) a PEG lipid in an amount from about 1.5-3.5 mol % of the lipid component;

wherein the ionizable lipid is a compound of Formula (I)



(I),

wherein

X^1 is O, NH, or a direct bond;

X^2 is C_{2-3} alkylene;

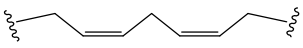
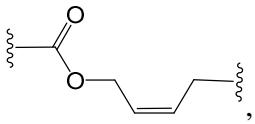
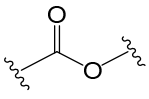
R^3 is C_{1-3} alkyl;

R^2 is C_{1-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 2-3 carbon atoms of X^2 form a 5- or 6-membered ring, or

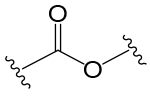
R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-membered ring;

Y^1 is C_{6-10} alkylene;

Y^2 is selected from , , and ;

R^4 is C_{4-11} alkyl;

Z^1 is C_{2-5} alkylene;

Z^2 is  or absent;

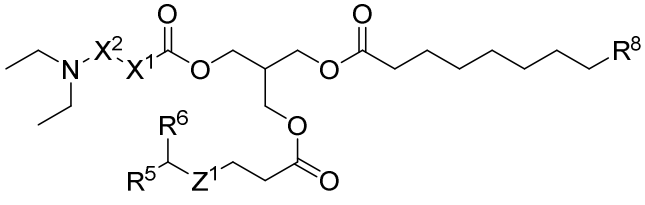
R^5 is C_{6-8} alkyl, C_{5-10} alkoxy (e.g., -OC_{5-10} haloalkyl), -O(C_{2-3} alkyl)OC_{6-10}alkyl, -OC_{6-10} alkenyl (e.g., -OC_{6-10} branched alkenyl), or -OC_{6-10} alkynyl, and

R^6 is C_{6-8} alkyl, C_{5-10} alkoxy (e.g., -OC_{5-10} haloalkyl), -O(C_{2-3} alkyl)OC_{6-10}alkyl, -OC_{6-10} alkenyl (e.g., -OC_{6-10} branched alkenyl), or -OC_{6-10} alkynyl, or

R^5 taken together with R^6 form a 6-member cyclic acetal substituted by geminal C_{6-8} alkyl;

or a salt thereof.

2. The lipid composition of claim 1, wherein the ionizable lipid is a compound of Formula (II)



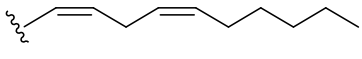
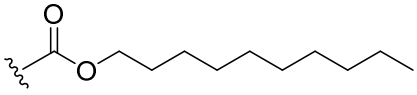
(II),

wherein

X^1 is O, NH, or a direct bond;

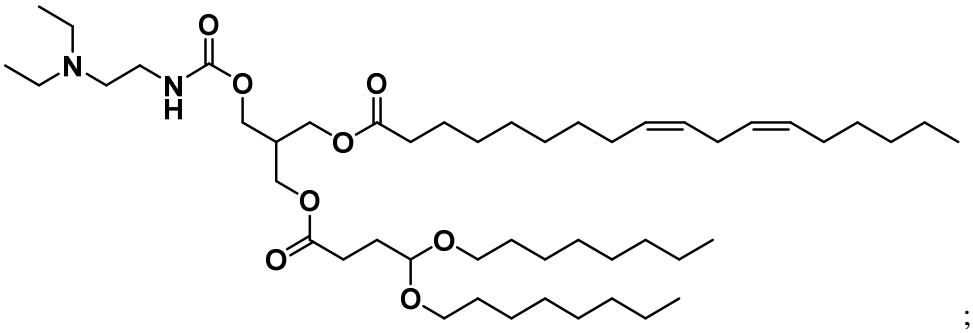
X^2 is C_{2-3} alkylene;

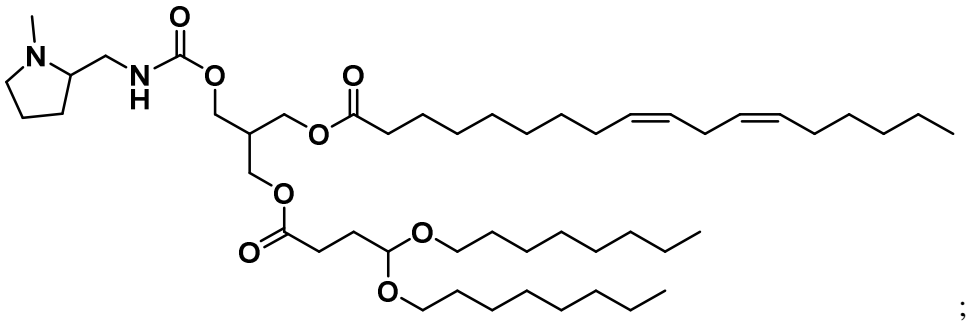
Z^1 is C_3 alkylene and R^5 and R^6 are each C_6 alkyl, or Z^1 is a direct bond and R^5 and R^6 are each C_8 alkoxy; and

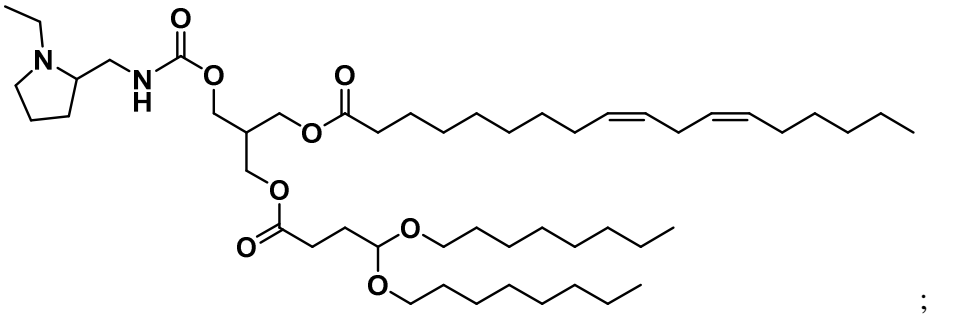
R^8 is  or ;

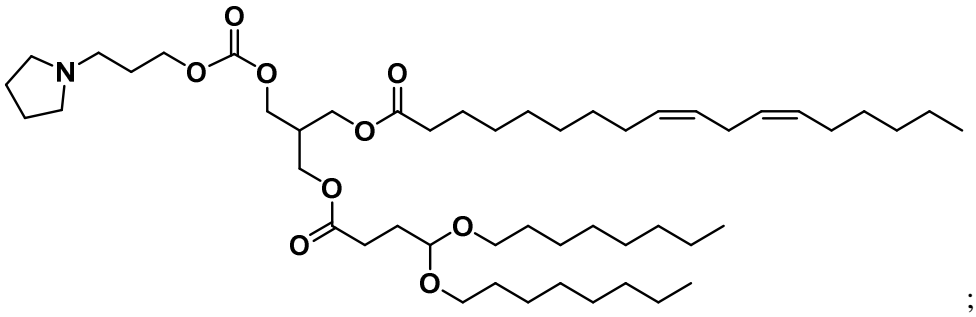
or a salt thereof.

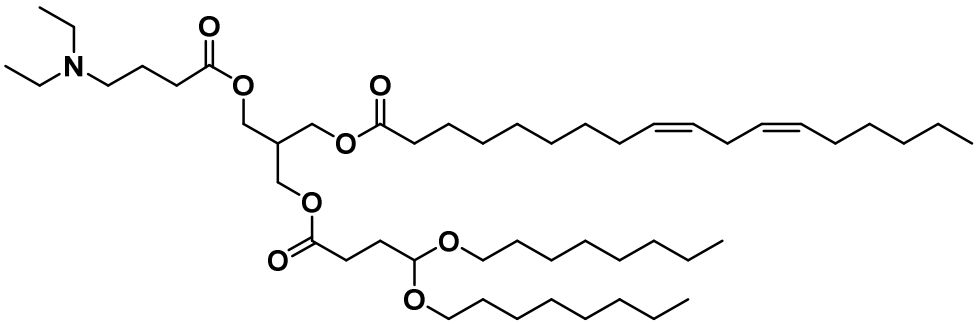
3. The lipid composition of claim 1, wherein the ionizable lipid is

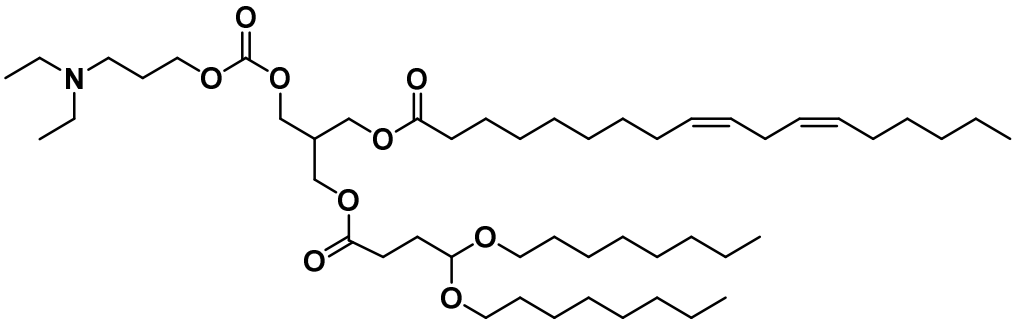

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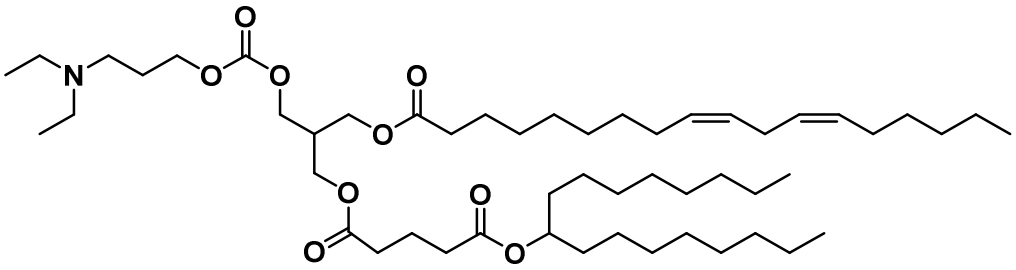

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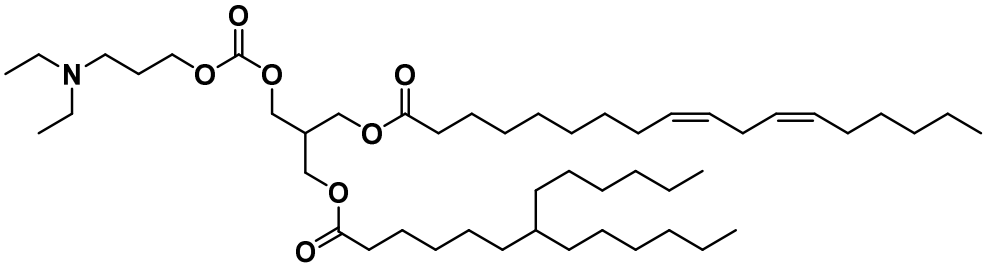

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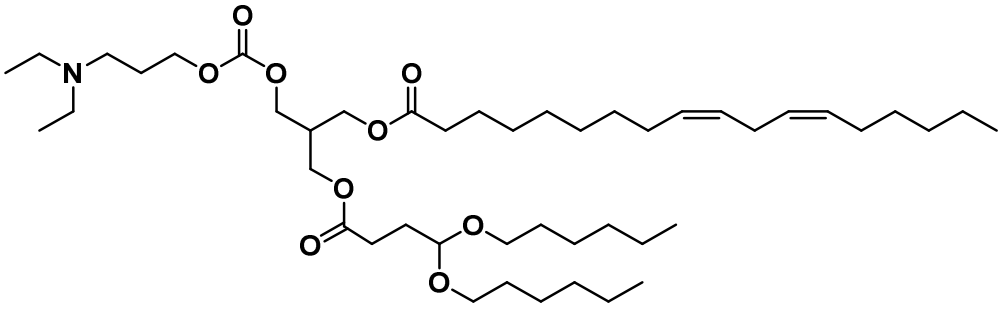

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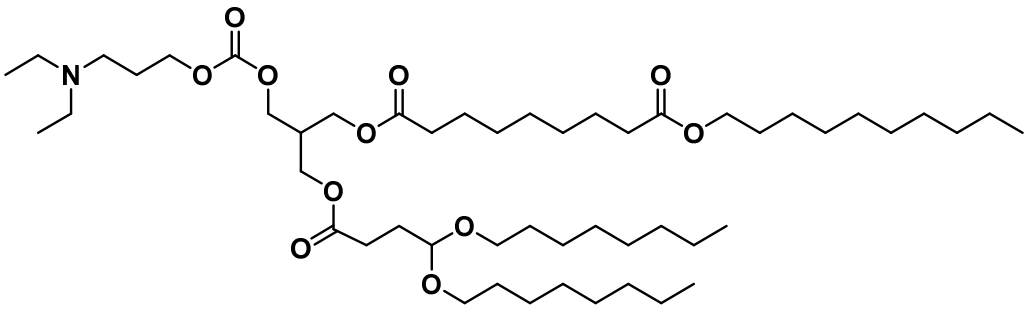

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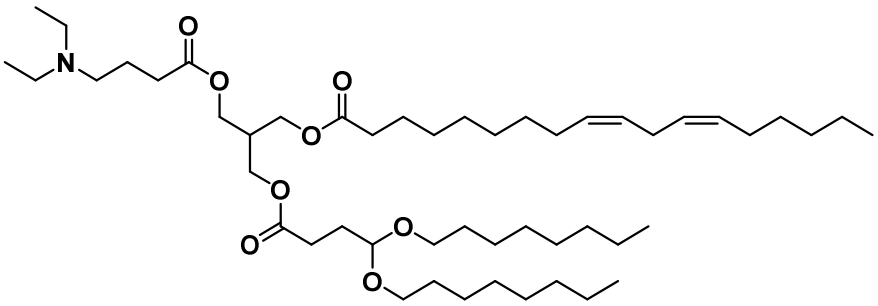

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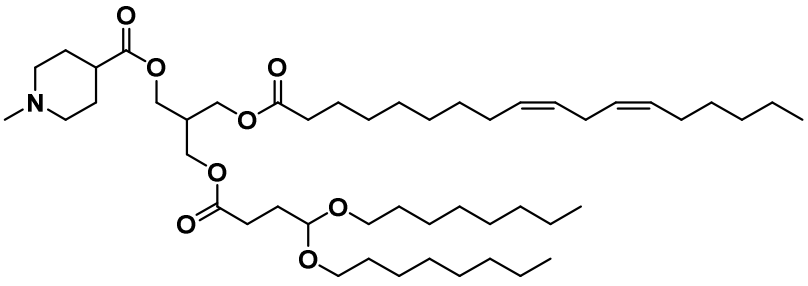

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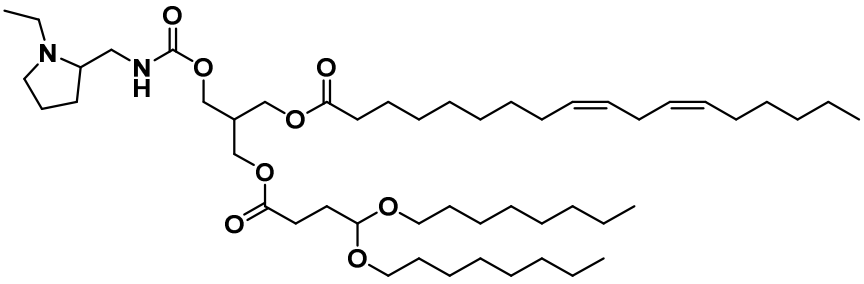
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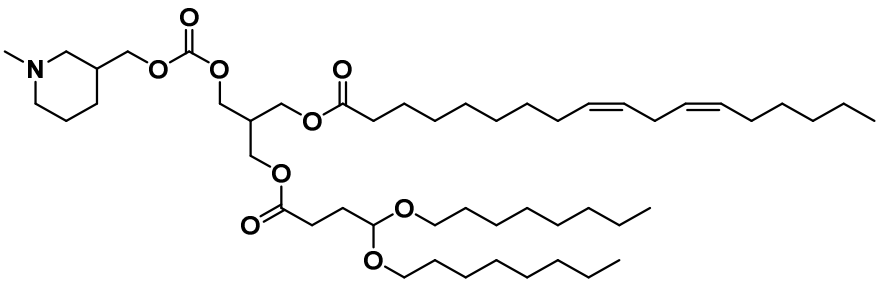
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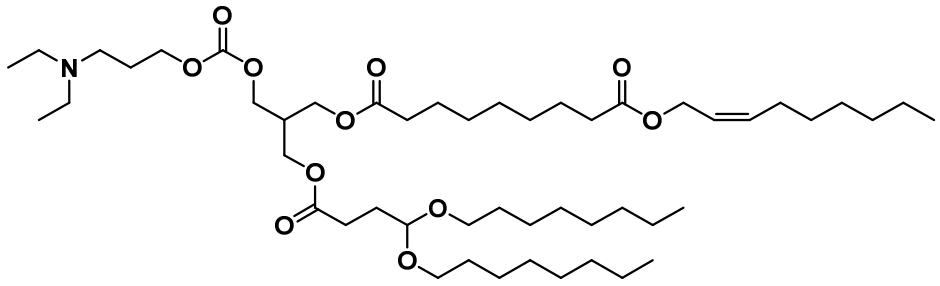
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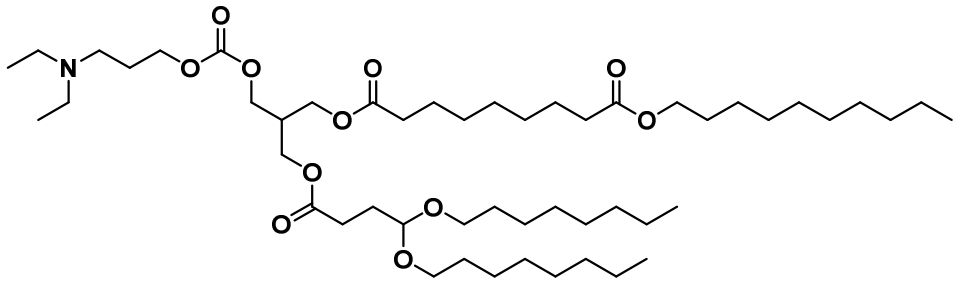
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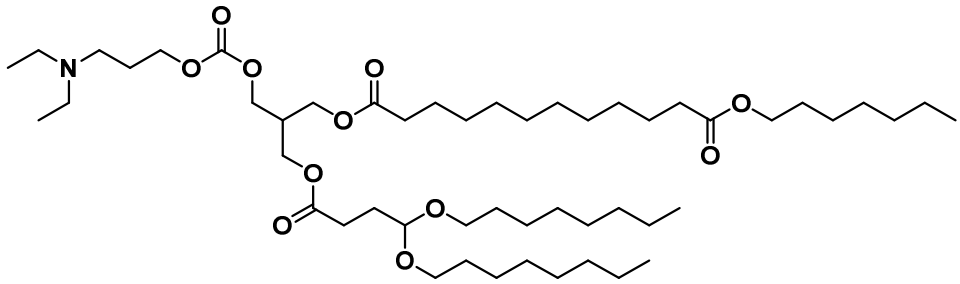
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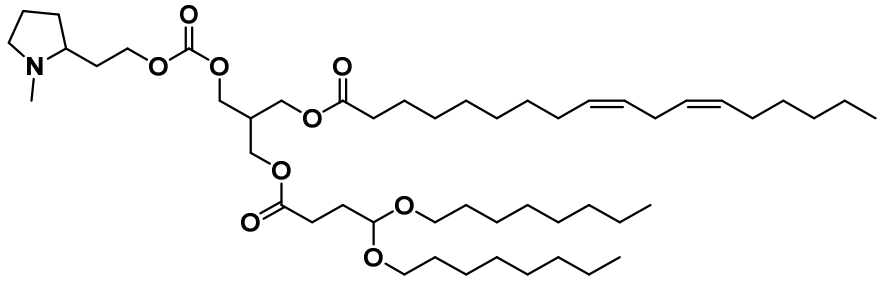
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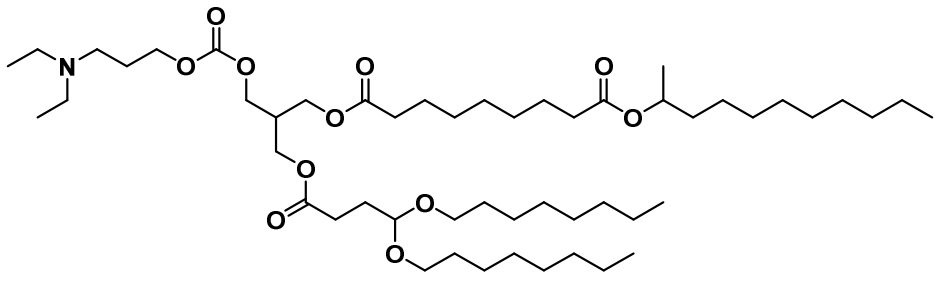
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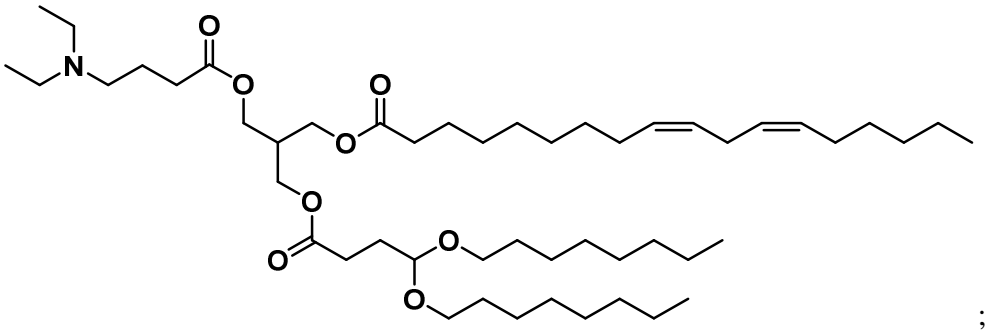
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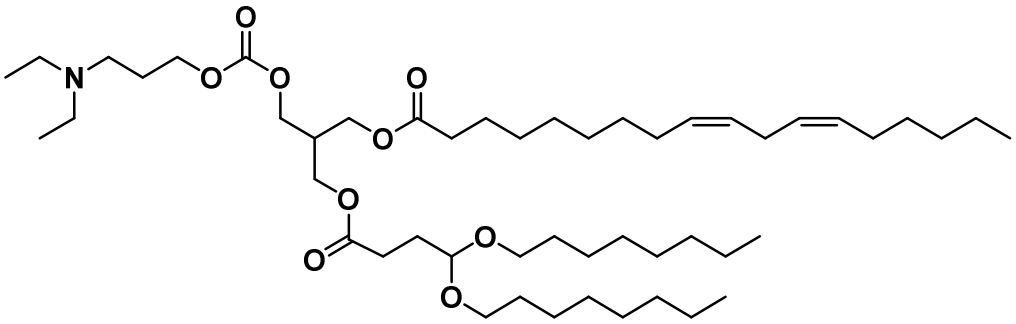


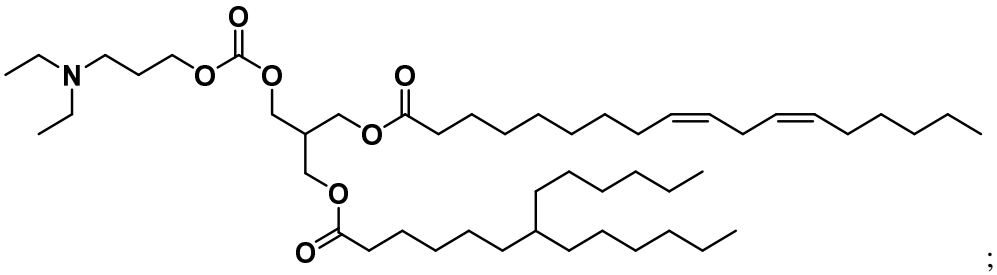
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or a salt thereof.

4. The lipid composition of any one of the preceding claims, wherein the ionizable lipid is

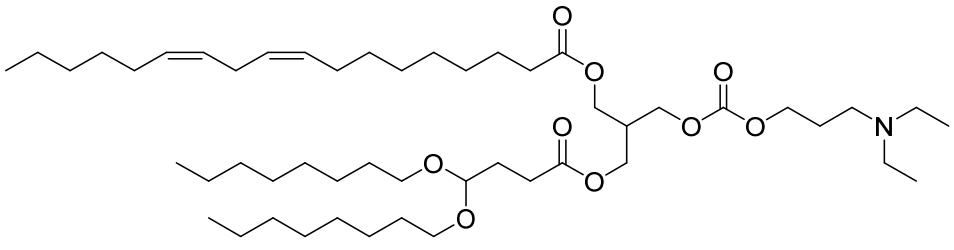

;


; or


;

or a salt thereof.

5. The lipid composition of any one of the preceding claims, wherein the ionizable lipid is


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or a salt thereof.

6. The lipid composition of any one of the preceding claims, wherein the neutral lipid is DSPC or DPME.

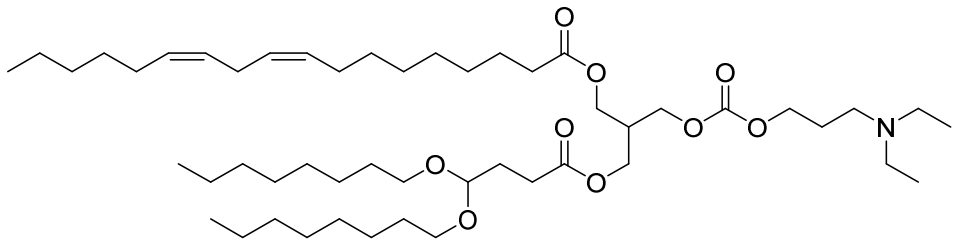
7. The lipid composition of any one of the preceding claims, wherein the helper lipid is selected from cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate.

8. The lipid composition of any one of the preceding claims, wherein the PEG lipid comprises dimyristoylglycerol (DMG).

9. The lipid composition of any one of the preceding claims, wherein the PEG lipid comprises PEG-2k.

10. The lipid composition of any one of the preceding claims, wherein the PEG lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000.

11. The lipid composition of any one of the preceding claims, wherein the ionizable lipid is



; the neutral lipid is DSPC; the helper lipid is cholesterol; and the PEG lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000.

12. The lipid composition of any one of the preceding claims, wherein the amount of the ionizable lipid is from about 29-38 mol % of the lipid component.

13. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is from about 30-43 mol % of the lipid component.

14. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is from about 25-34 mol % of the lipid component.

15. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is about 33 mol % of the lipid component.

16. The lipid composition of any one of the preceding claims, wherein the amount of the neutral lipid is from about 11-20 mol % of the lipid component.

17. The lipid composition of any one of claims 1-15, wherein the amount of the neutral lipid is about 15 mol % of the lipid component.

18. The lipid composition of any one of the preceding claims, wherein the amount of the helper lipid is from about 43-65 mol % of the lipid component.

19. The lipid composition of any one of claims 1-17, wherein the amount of the helper lipid is about 49 mol % of the lipid component.

20. The lipid composition of any one of the preceding claims, wherein the amount of the PEG lipid is from about 2.0-3.5 mol % of the lipid component.

21. The lipid composition of any one of claims 1-19, wherein the amount of the PEG lipid is about 2.7 mol % of the lipid component.

22. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is from about 29-44 mol % of the lipid component; the amount of the neutral lipid is from about 11-28 mol % of the lipid component; the amount of the helper lipid is from about 28-55 mol % of the lipid component; and the amount of the PEG lipid is from about 2.3-3.5 mol % of the lipid component.

23. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is from about 29-38 mol % of the lipid component; the amount of the neutral lipid is

from about 11-20 mol % of the lipid component; the amount of the helper lipid is from about 43-55 mol % of the lipid component; and the amount of the PEG lipid is from about 2.3-2.7 mol % of the lipid component.

24. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is from about 25-34 mol % of the lipid component; the amount of the neutral lipid is from about 10-20 mol % of the lipid component; the amount of the helper lipid is from about 45-65 mol % of the lipid component; and the amount of the PEG lipid is from about 2.5-3.5 mol % of the lipid component.

25. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is about 30-43 mol % of the lipid component; the amount of the neutral lipid is about 10-17 mol % of the lipid component; the amount of the helper lipid is about 43.5-56 mol % of the lipid component; and the amount of the PEG lipid is about 1.5-3 mol % of the lipid component.

26. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is about 33 mol % of the lipid component; the amount of the neutral lipid is about 15 mol % of the lipid component; the amount of the helper lipid is about 49 mol % of the lipid component; and the amount of the PEG lipid is about 3 mol % of the lipid component.

27. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is about 32.9 mol % of the lipid component; the amount of the neutral lipid is about 15.2 mol % of the lipid component; the amount of the helper lipid is about 49.2 mol % of the lipid component; and the amount of the PEG lipid is about 2.7 mol % of the lipid component.

28. The lipid composition of any one of claims 1-11, wherein the amount of the ionizable lipid is about 35 mol % of the lipid component; the amount of the neutral lipid is about 15 mol % of the lipid component; the amount of the helper lipid is about 47.5 mol % of the

lipid component; and the amount of the PEG lipid is about 2.5 mol % of the lipid component.

29. The lipid composition of any one of the preceding claims, wherein the lipid composition is in the form of LNPs; and the LNPs have a polydispersity index of about 0.005 to about 0.75.

30. The lipid composition of any one of the preceding claims, wherein the N/P ratio of the lipid composition is from about 5 to about 7.

31. The lipid composition of any one of the preceding claims, wherein the biologically active agent comprises a nucleic acid component; and the nucleic acid component comprises an mRNA encoding an RNA-guided DNA-binding agent.

32. The lipid composition of claim 31, wherein the mRNA comprises a Cas9 nuclease mRNA.

33. The lipid composition of claim 31 or 32, wherein the nucleic acid component comprises a guide RNA nucleic acid.

34. The lipid composition of claim 33, wherein the guide RNA nucleic acid is or encodes a dual-guide RNA (dgRNA) or single-guide RNA (sgRNA).

35. The lipid composition of any one of claims 31-34, wherein the nucleic acid component comprises a guide RNA nucleic acid; the mRNA comprises a Class 2 Cas nuclease mRNA; and the ratio of the mRNA to the guide RNA nucleic acid is from about 2:1 to 1:4 by weight.

36. The lipid composition of claim 35, wherein the ratio of the guide RNA nucleic acid to the Class 2 Cas nuclease mRNA is about 1:1 by weight.

37. The lipid composition of any one of the preceding claims, wherein the lipid composition is an LNP composition.

38. An in vitro method of gene editing, an in vitro method of cleaving a DNA, or a method of delivering a biologically active agent to a cell in vitro comprising contacting a cell with a lipid composition of any one of the preceding claims.

39. The method of claim 38, wherein the cell is a stem cell.

40. The method of claim 38 or 39, wherein the cell is an immune cell.

41. A method of producing multiple genome edits in a cell or a population of cells, comprising

contacting the cell or the population of cells in vitro with at least a first lipid composition of any one of claims 1-37 and a second lipid composition of any one of claims 1-37,

wherein the biologically active agent of the first lipid composition comprises a first guide RNA (gRNA) directed to a first target sequence and optionally a nucleic acid genome editing tool, and

the biologically active agent of the second lipid composition comprises a second gRNA directed to a second target sequence and optionally a nucleic acid genome editing tool,

thereby producing multiple genome edits in the cell or the population of cells.

42. The method of claim 41, wherein the cell is a stem cell, or the population of cells is a population of stem cells.

43. The method of claim 41 or 42, wherein the cell is an immune cell, or the population of cells is a population of immune cells.