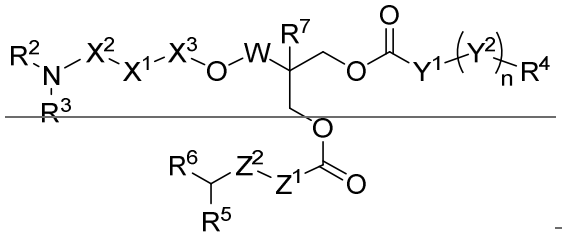
What is claimed isCLAIMS:

1. A compound of Formula II



(II),

wherein

X^1 is O, NR^1, or a direct bond,

X^2 is C_{2-5} alkylene,

X^3 is C(=O) or a direct bond,

R^1 is H or Me,

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

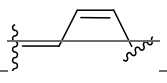
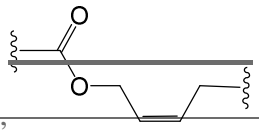
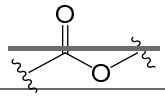
R^2 is C_{1-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or

X^1 is NR^1, R^1 and R^2 taken together with the nitrogen atoms to which they are attached 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-, 6-, or 7-membered ring,

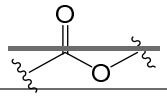
Y^1 is C_{2-12} alkylene,

Y^2 is selected from  (in either orientation),  (in either orientation), and  (in either orientation),

n is 0 to 3,

R^4 is C_{1-15} alkyl,

Z^1 is C_{1-6} alkylene or a direct bond,

Z^2 is  (in either orientation) or absent, provided that if Z^1 is a direct bond, Z^2 is absent;

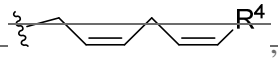
R^5 is C_{5-9} alkyl or C_{6-10} alkoxy,

R^6 is C_{5-9} alkyl or C_{6-10} alkoxy,

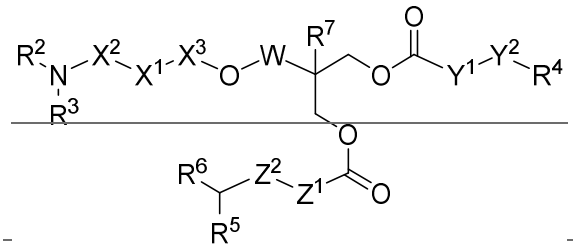
W is methylene or a direct bond, and

R^7 is H or Me,

or a salt thereof,

provided that if R^3 and R^2 are C_2 alkyls, X^1 is O, X^2 is linear C_3 alkylene, X^3 is C(=O), Y^1 is linear C_6 alkylene, $(Y^2)_n$ R^4 is , R^4 is linear C_5 alkyl, Z^1 is C_2 alkylene, Z^2 is absent, W is methylene, and R^7 is H, then R^5 and R^6 are not C_8 alkoxy.

2. The compound of claim 1, wherein the compound is a compound of Formula I



(I),

wherein

X^1 is O, NR^1, or a direct bond,

X^2 is C_{2-5} alkylene,

X^3 is C(=O) or a direct bond,

R^1 is H or Me,

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

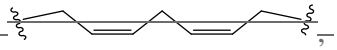
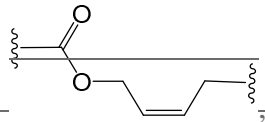
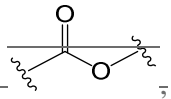
R^2 is C_{1-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or

X^1 is NR^1, R^1 and R^2 taken together with the nitrogen atoms to which they are attached form a 5- or 6-membered ring, or

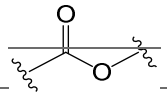
R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-, 6-, or 7-membered ring,

Y^1 is C_{2-12} alkylene,

~~Y^2 is selected from~~ , , ~~and~~ ,

~~R^4 is C_{3-15} alkyl,~~

~~Z^1 is C_{1-6} alkylene or a direct bond,~~

~~Z^2 is~~  ~~(in either orientation) or absent, provided that if Z^1 is a direct bond, Z^2 is absent;~~

~~R^5 is C_{5-9} alkyl or C_{6-10} alkoxy,~~

~~R^6 is C_{5-9} alkyl or C_{6-10} alkoxy,~~

~~W is methylene or a direct bond, and~~

~~R^7 is H or Me,~~

~~or a salt thereof,~~

~~provided that if R^3 and R^2 are C_2 alkyls, X^1 is O, X^2 is linear C_3 alkylene, X^3 is C(=O), Y^1 is linear C_6 alkylene, Y^2 is~~ , ~~R^4 is linear C_4 alkyl, Z^1 is C_2 alkylene, Z^2 is absent, W is methylene, and R^7 is H, then R^5 and R^6 are not C_8 alkoxy.~~

~~3. The compound of claim 1 or claim 2, wherein X^2 is linear C_2 alkylene.~~

~~4. The compound of claim 1 or claim 2, wherein X^2 is linear C_3 alkylene.~~

~~5. The compound of claim 1 or claim 2, wherein X^2 is linear C_4 alkylene.~~

~~6. The compound of any one of the preceding claims, wherein R^3 is C_1 alkyl.~~

~~7. The compound of any claims 1-5, wherein R^3 is C_2 alkyl.~~

~~8. The compound of any one of the preceding claims, wherein R^2 is C_1 alkyl.~~

~~9. The compound of any one claims 1-7, wherein R^2 is C_2 alkyl.~~

~~10. The compound of claims 1-7, wherein R^2 taken together with the nitrogen atom and 1-2 carbon atoms of X^2 form a 5-membered ring.~~

~~11. The compound of claims 1-7, wherein R^2 taken together with the nitrogen atom and 1-3 carbon atoms of X^2 form a 6-membered ring.~~

~~12. The compound of claims 1-5, wherein R^2 and R^3 taken together with the nitrogen atom form a 5-membered ring.~~

~~13. The compound of any one of the preceding claims, wherein X^1 is NH.~~

~~14. The compound of claims 1-12, wherein X^1 is a direct bond.~~

~~15. The compound of any one of the preceding claims, wherein Y^1 is linear C_{3-10} alkylene.~~

~~16. The compound of any one of the preceding claims, wherein Y^1 is linear C_{4-8} alkylene.~~

~~17. The compound of any one of the preceding claims, wherein Y^1 is linear C_{5-7} alkylene.~~

~~18. The compound of any one of the preceding claims, wherein R^4 is linear C_{4-14} alkyl.~~

~~19. The compound of any one of the preceding claims, wherein R^4 is linear C_{6-12} alkyl.~~

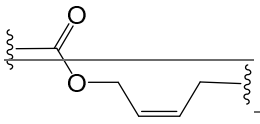
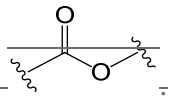
~~20. The compound of any one of the preceding claims, wherein Z^1 is linear C_{2-4} alkylene.~~

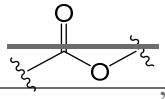
~~21. The compound of any one of the preceding claims, wherein R^5 and R^6 are each independently linear C_{5-9} alkyl.~~

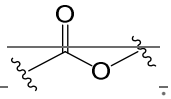
~~22. The compound of any one of the preceding claims, wherein R^5 and R^6 are each independently linear C_{6-8} alkyl.~~

~~23. The compound of any one of the preceding claims, wherein R^5 and R^6 are each independently linear C_{7-9} alkoxy.~~

24. The compound of any one of the preceding claims, wherein Y^2 is

 or .

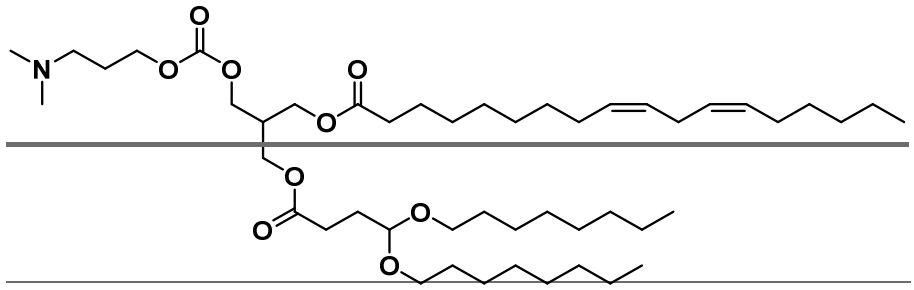
25. The compound of any one of the preceding claims, wherein Y^1 is linear C_7 alkylene, Y^2 is , n = 1, and R^4 is linear C_{10} alkyl.

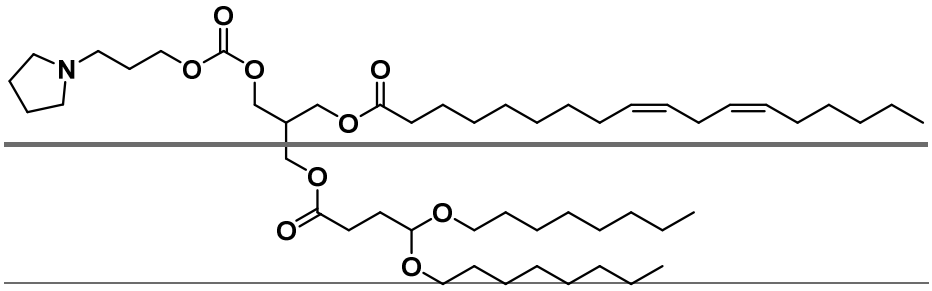
26. The compound of any one of the preceding claims, wherein Z^2 is .

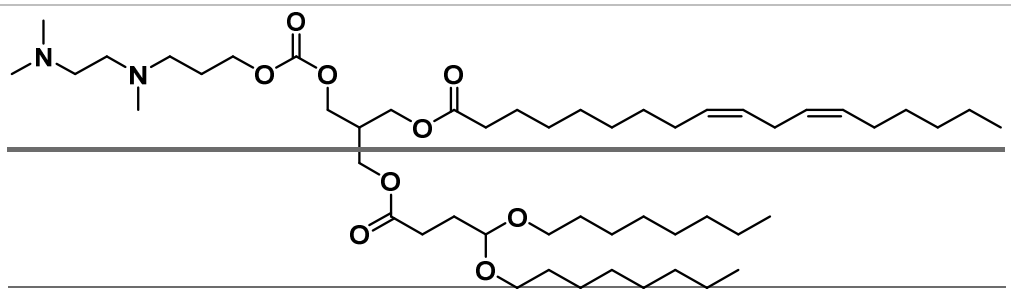
27. The compound of any one of the preceding claims, wherein Z^1, Z^2, and R^5 are selected to form a linear chain of 6-18 atoms, including the carbon and oxygen atoms of the ester and the acetal.

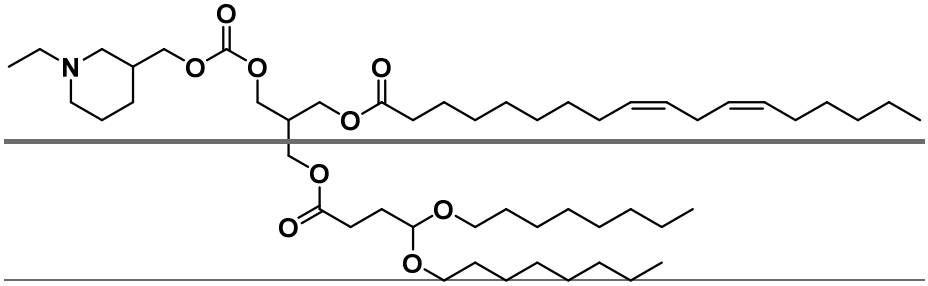
28. The compound of any one of the preceding claims, wherein Y^1, Y^2, and R^4 are selected to form a linear chain of 14-24 atoms, including the carbon and oxygen atoms of the ester(s), if present.

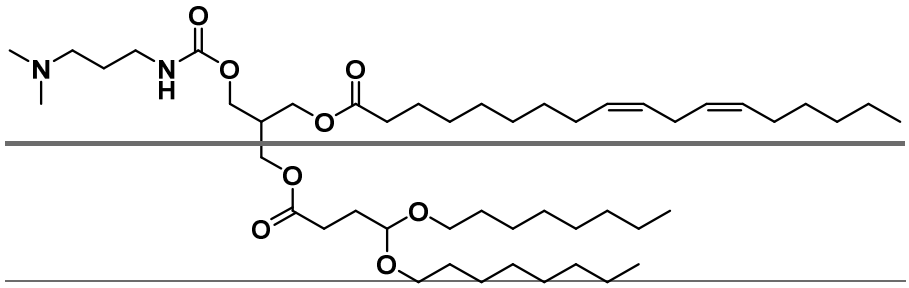
29. The compound of claim 1, wherein the compound is selected from:

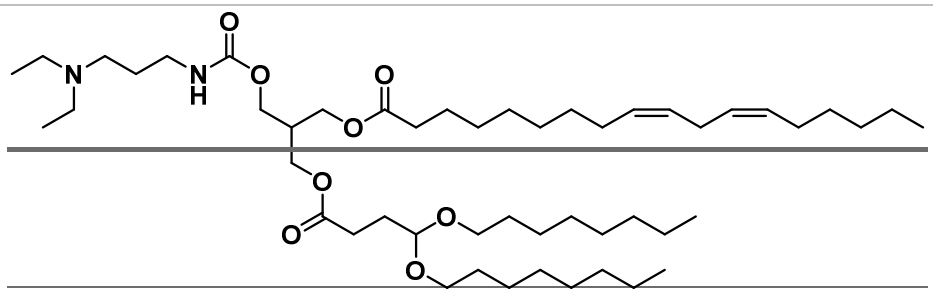


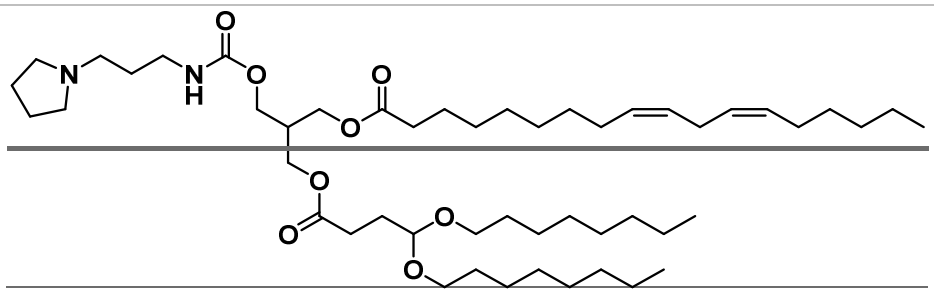


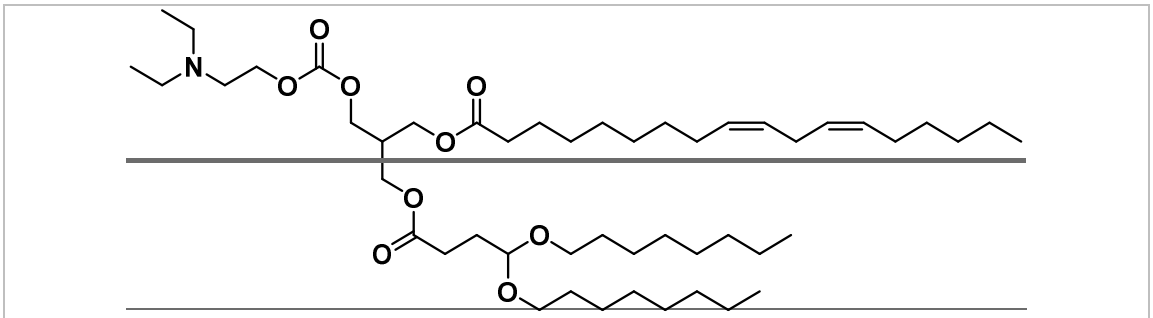


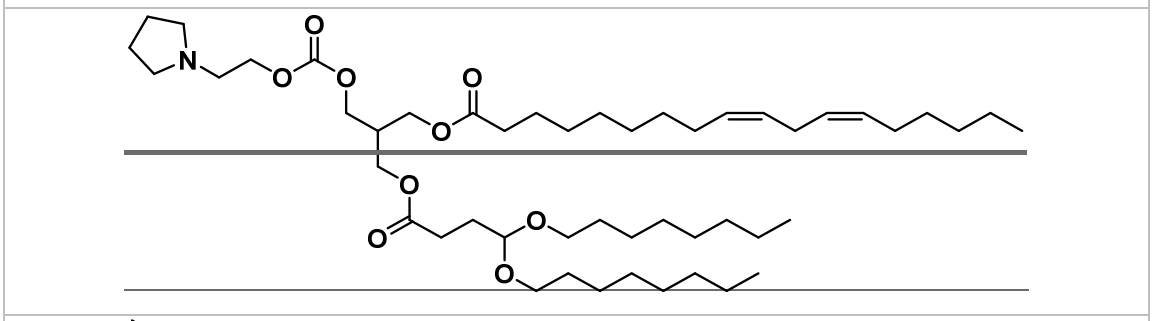


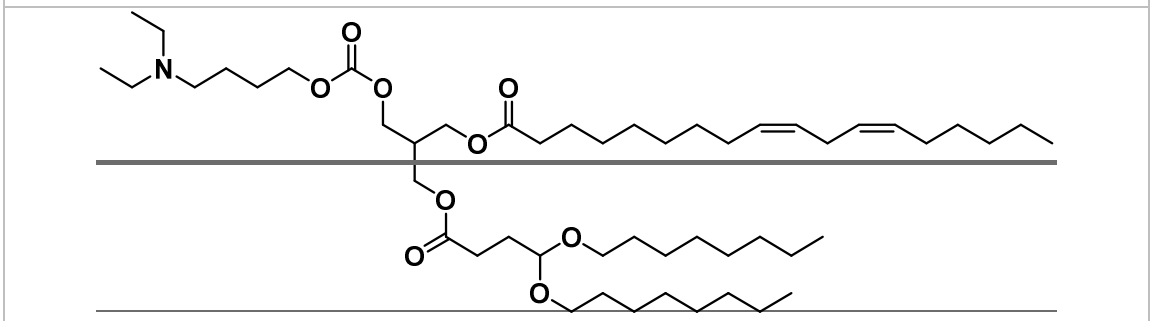


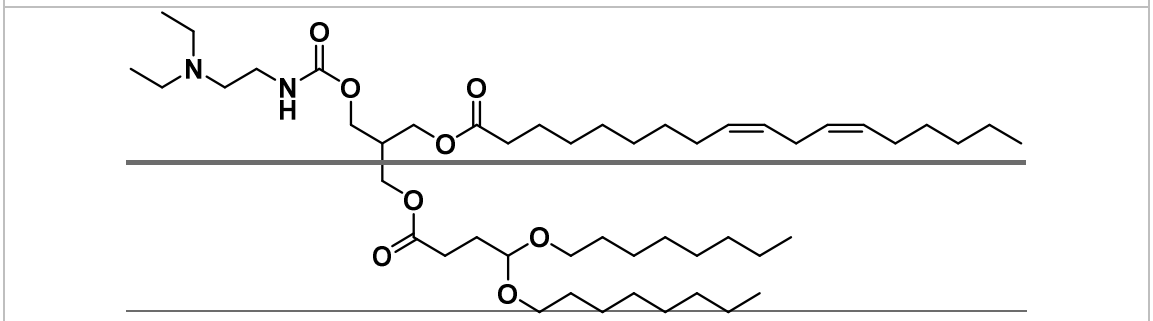


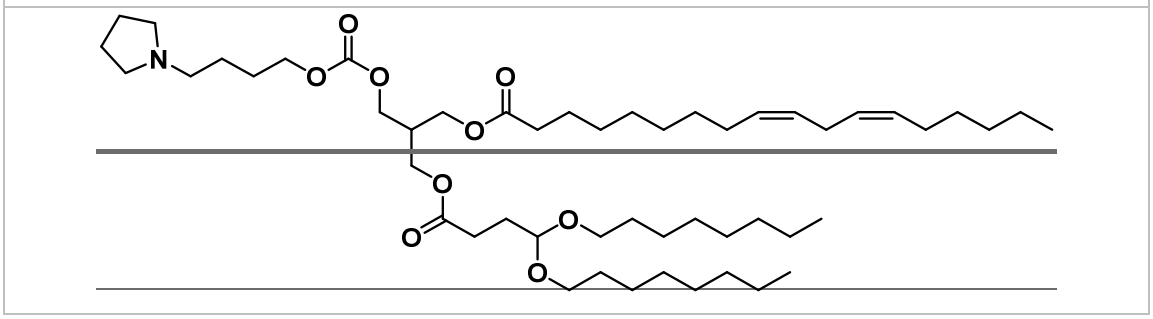


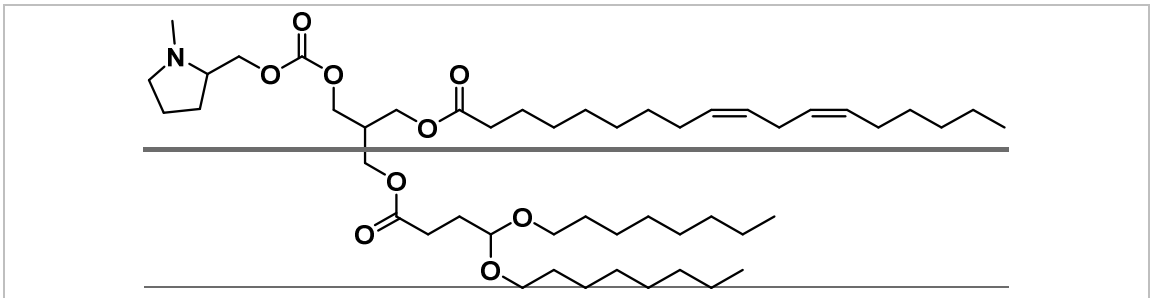


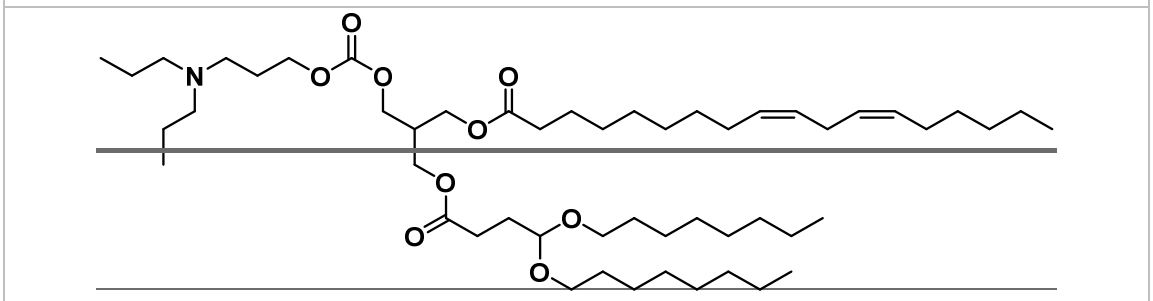


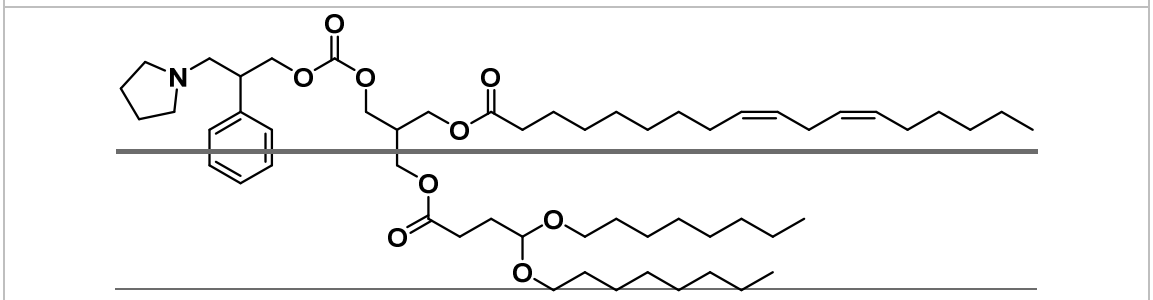


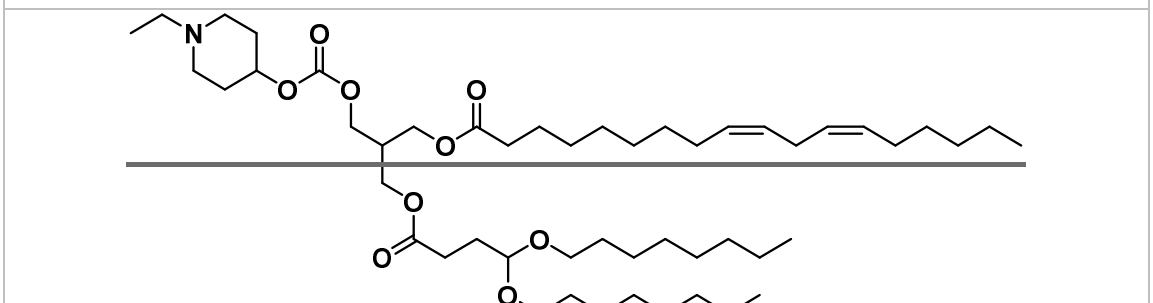


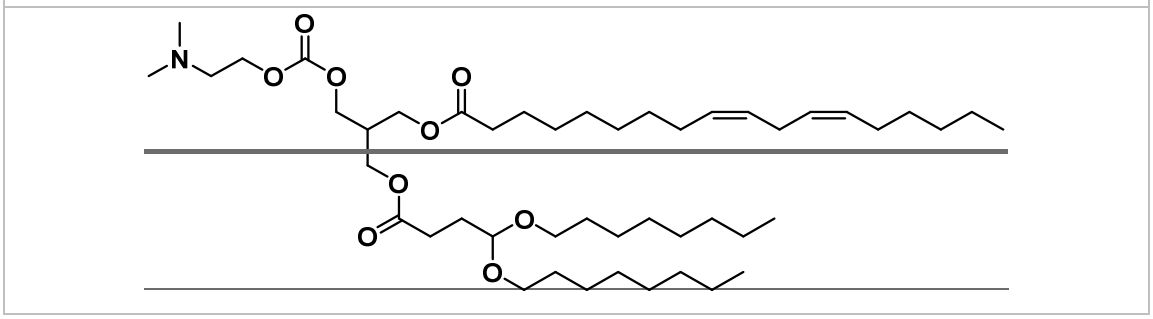


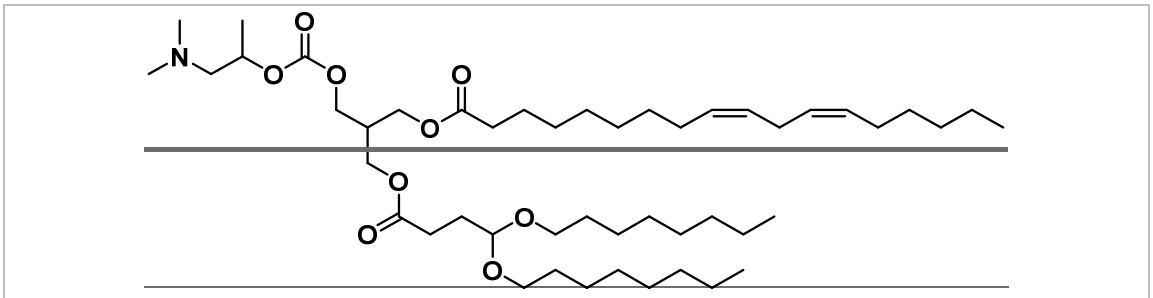


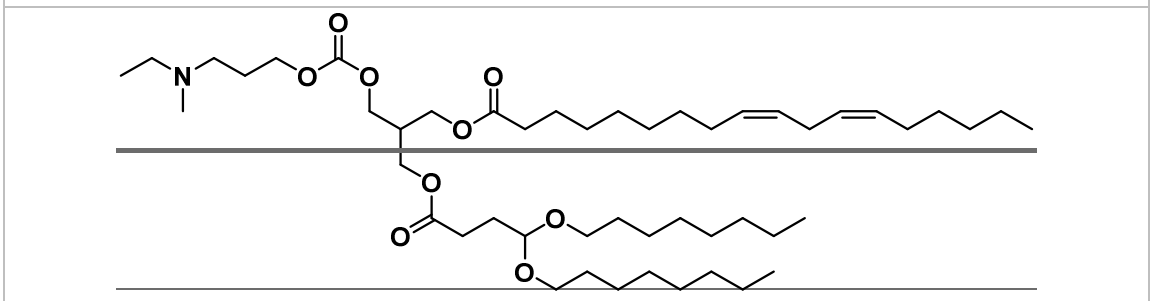


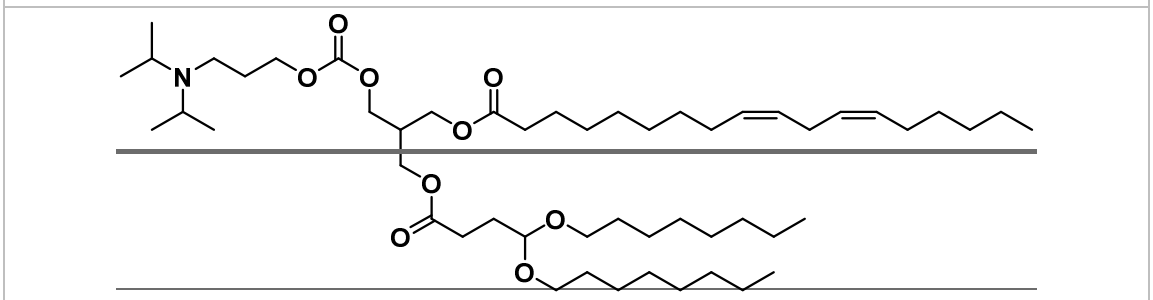


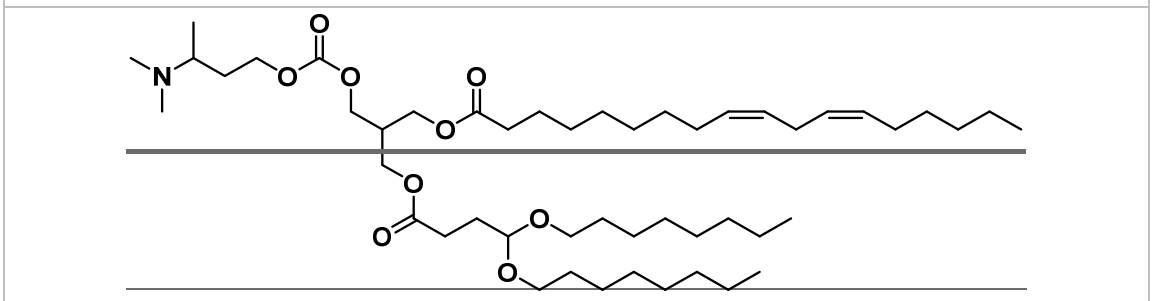


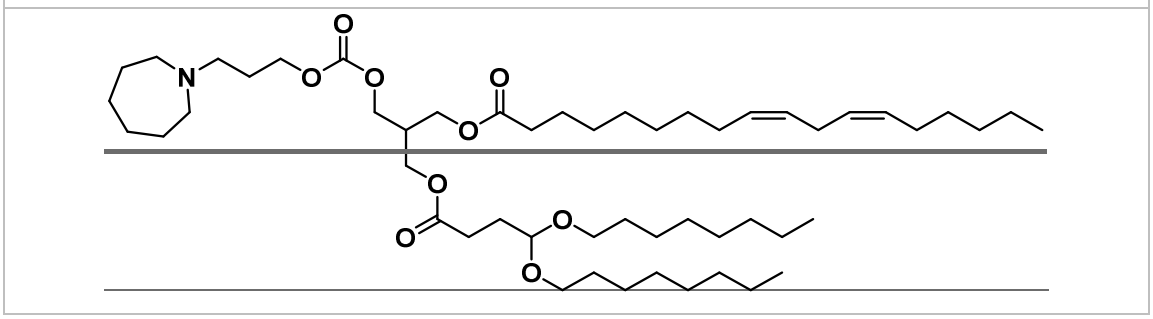


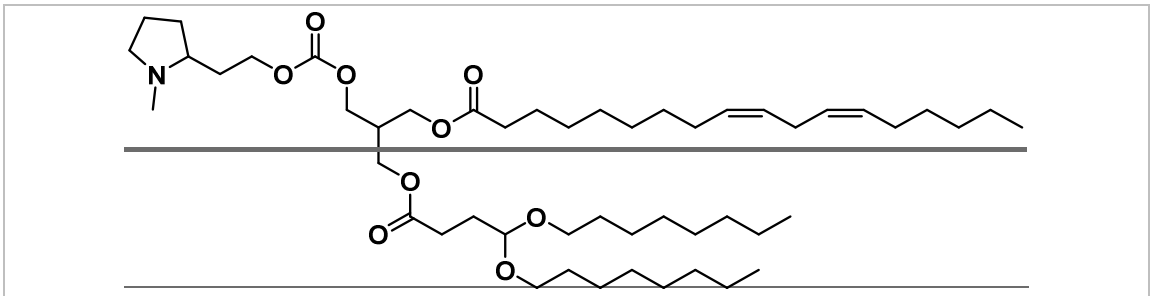


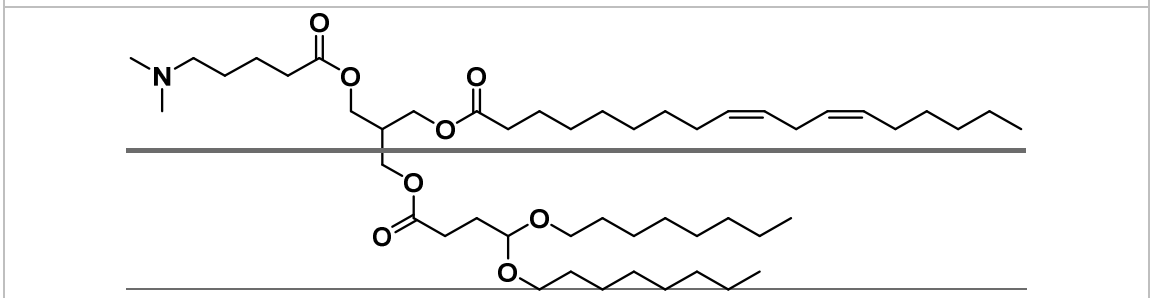


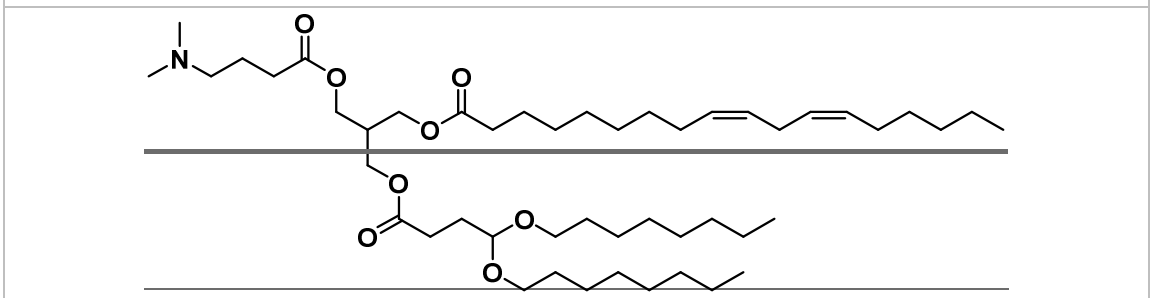


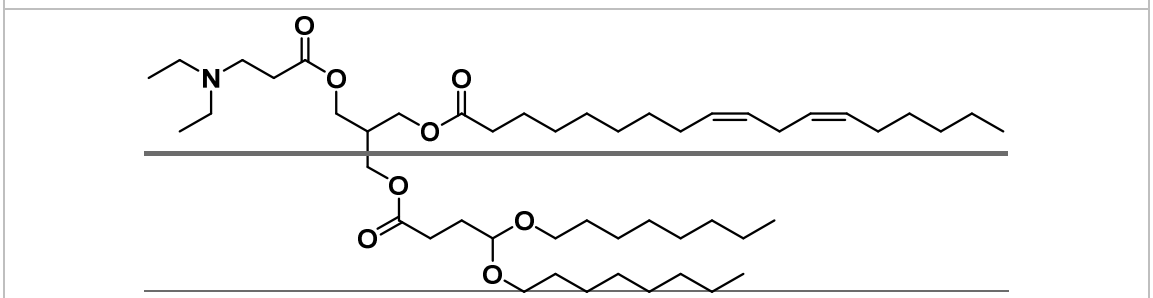


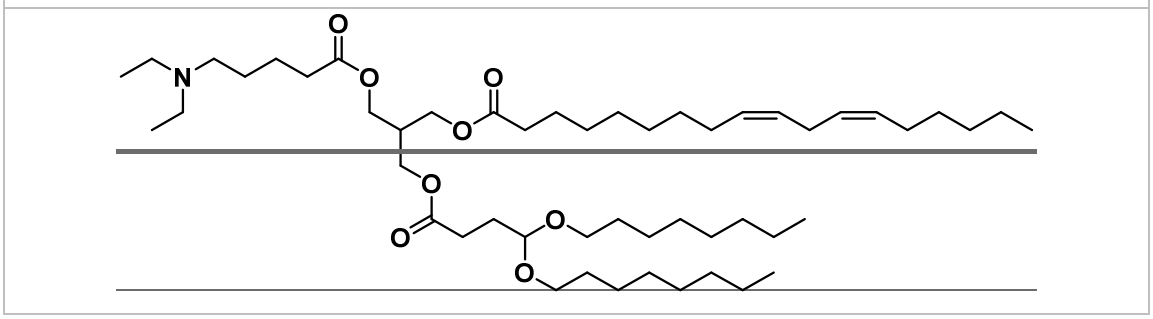


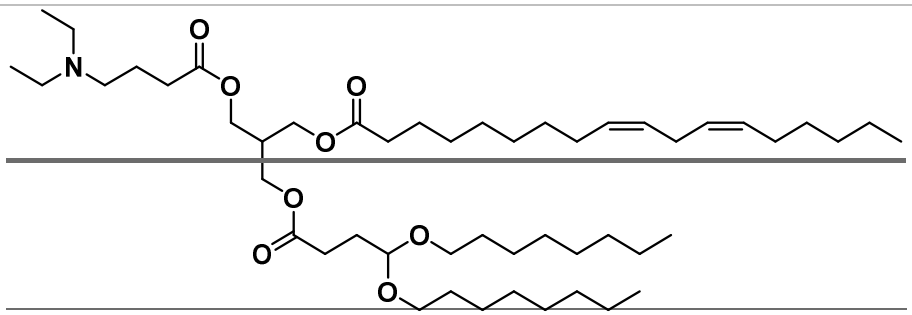


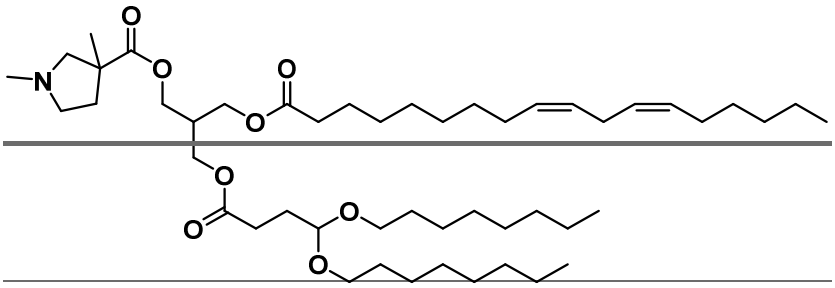


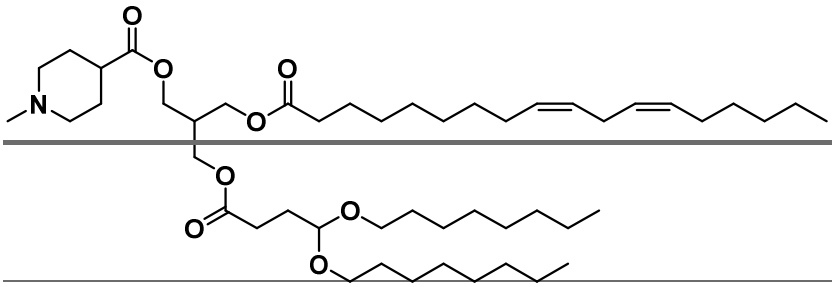


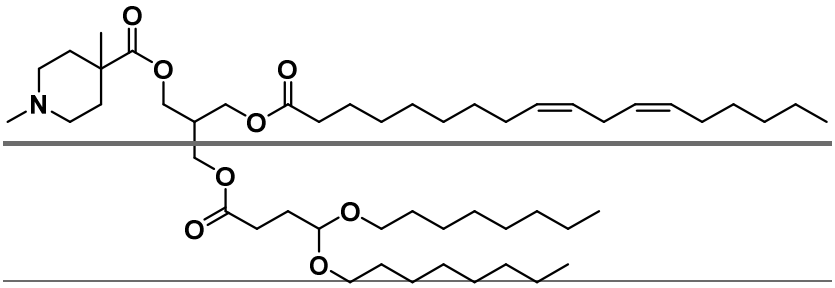


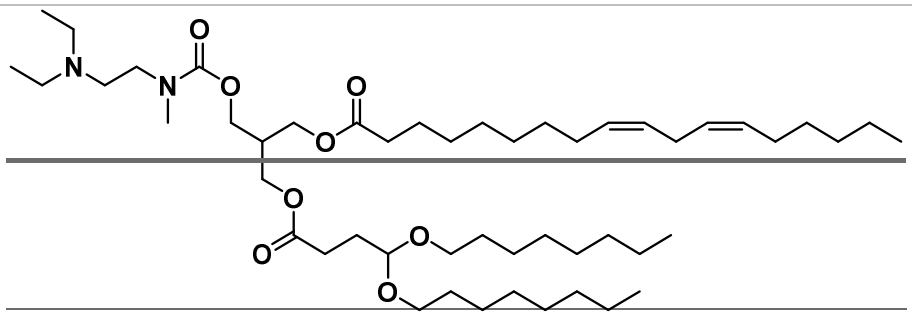


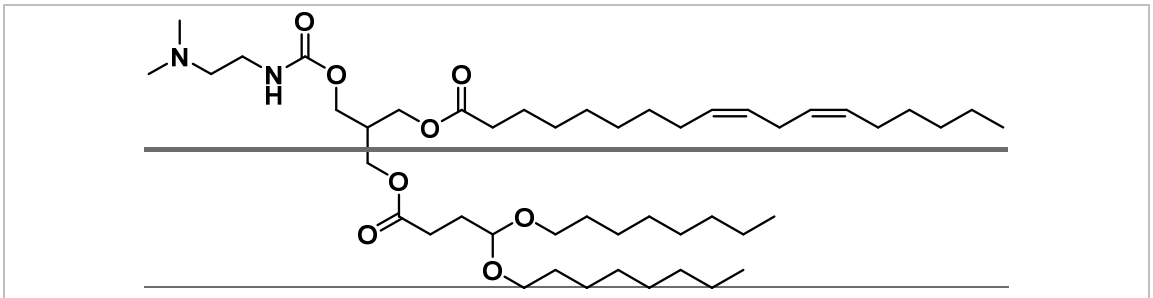


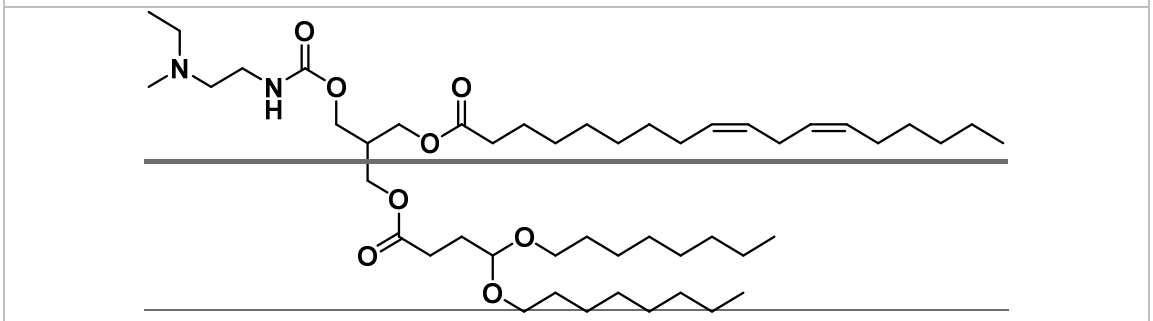


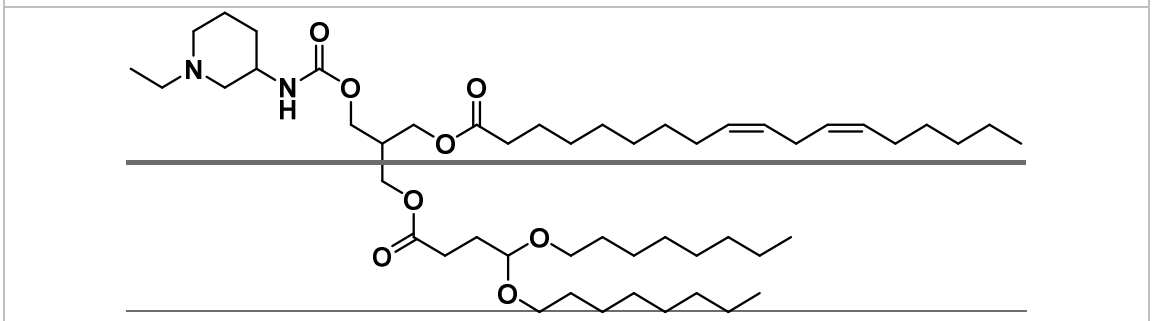


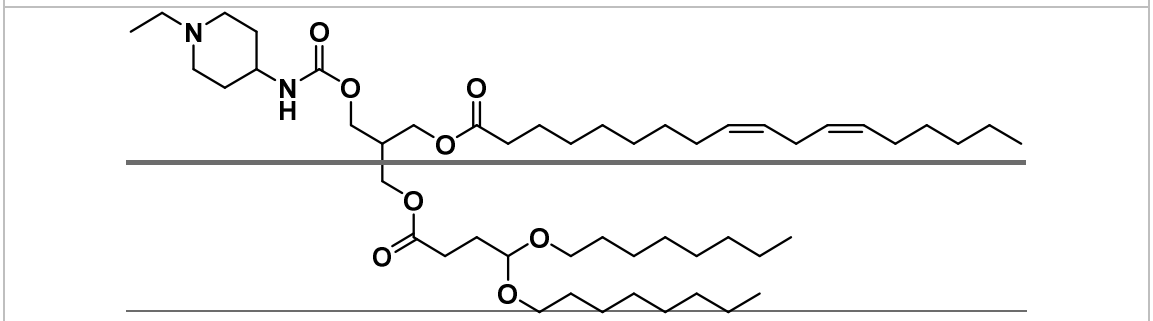


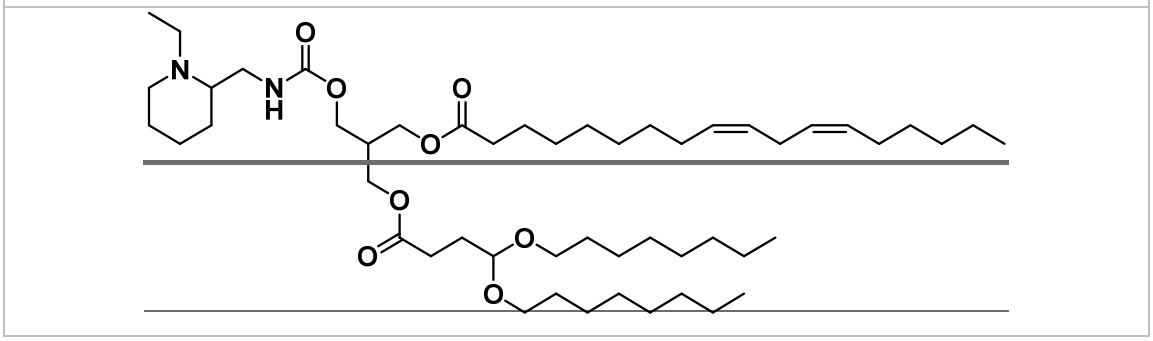


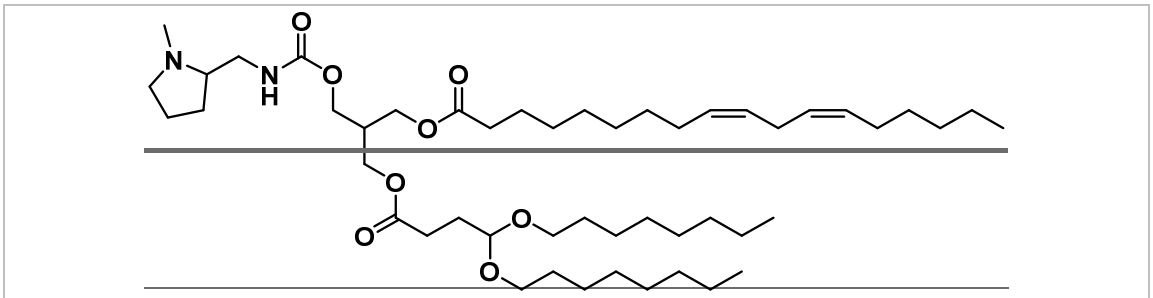


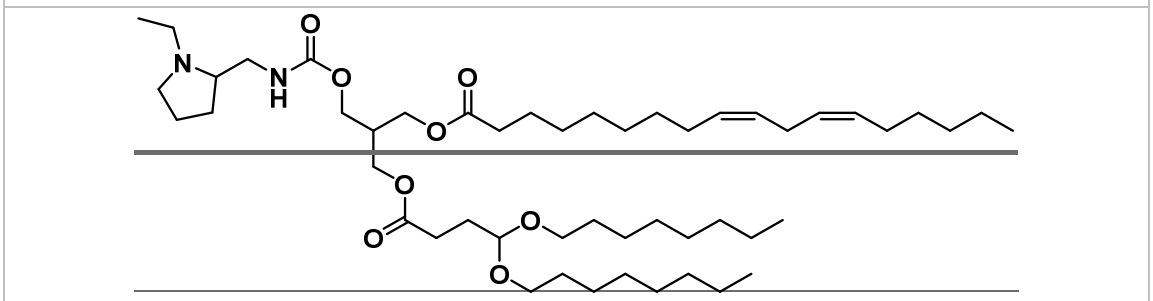


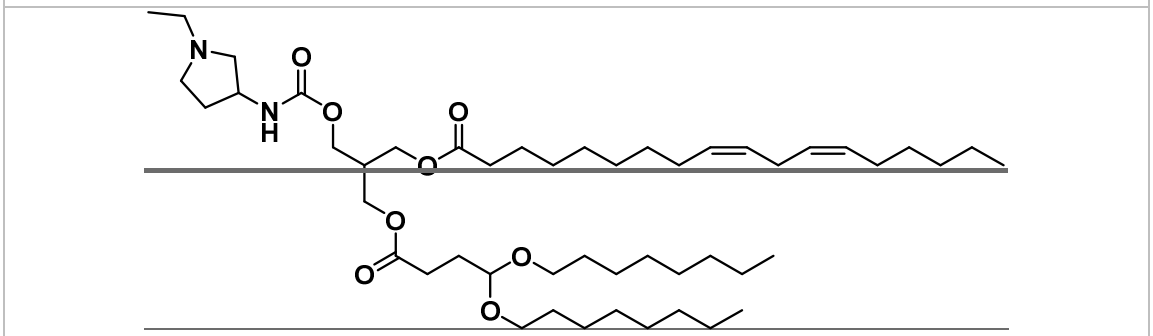


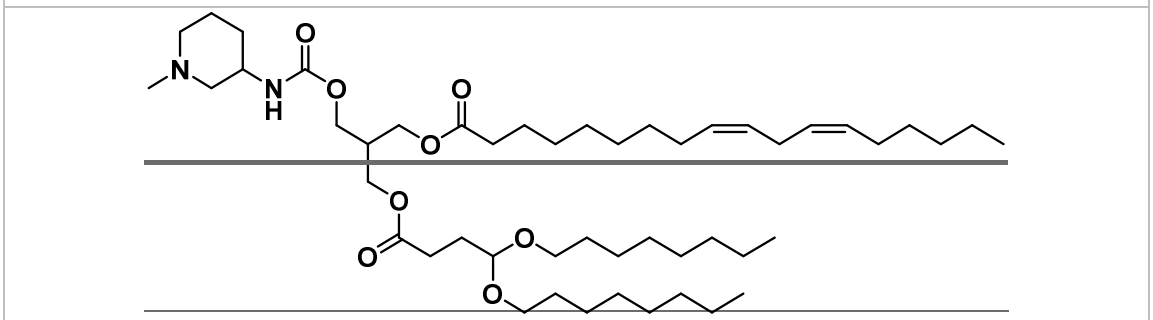


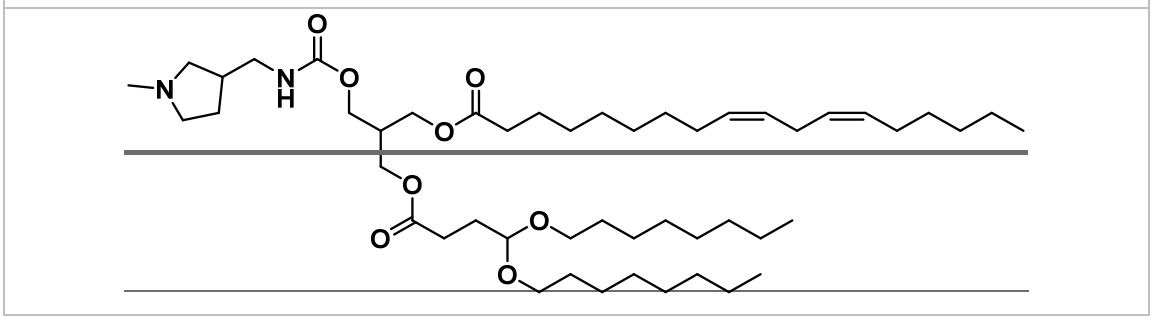


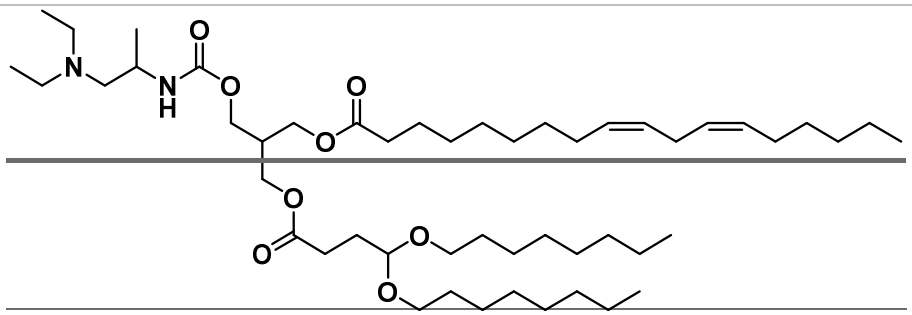


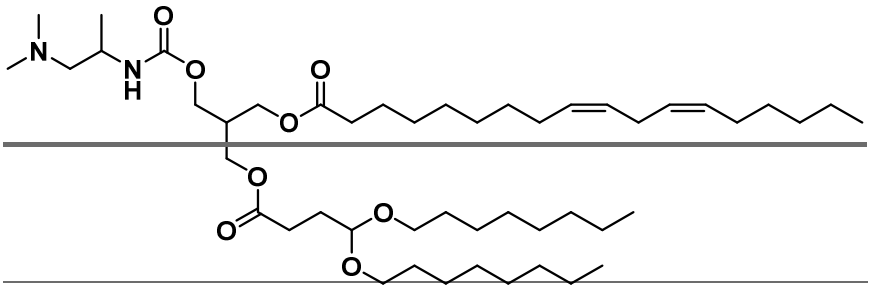


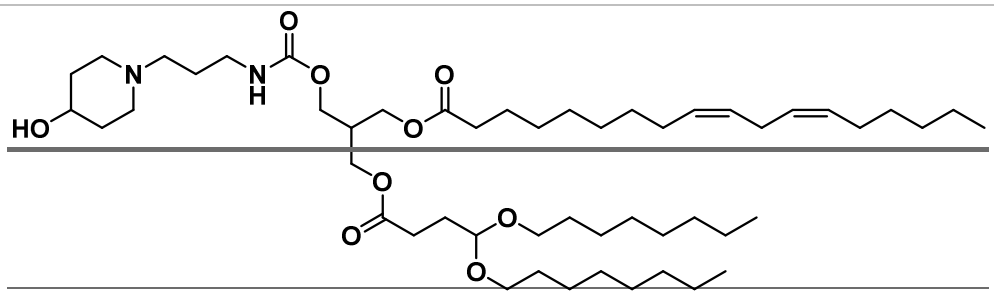


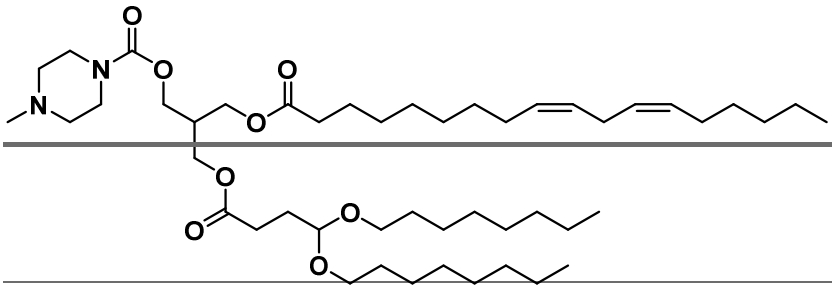


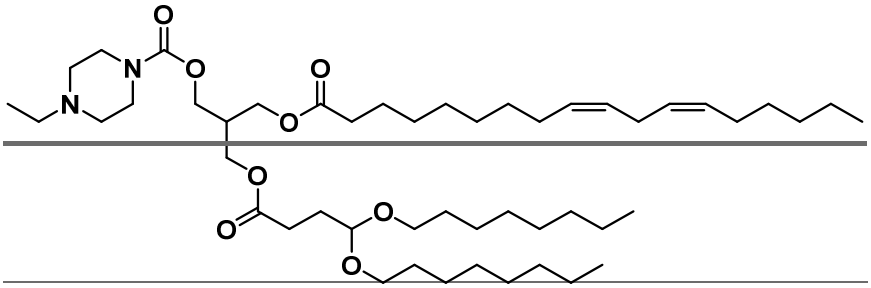


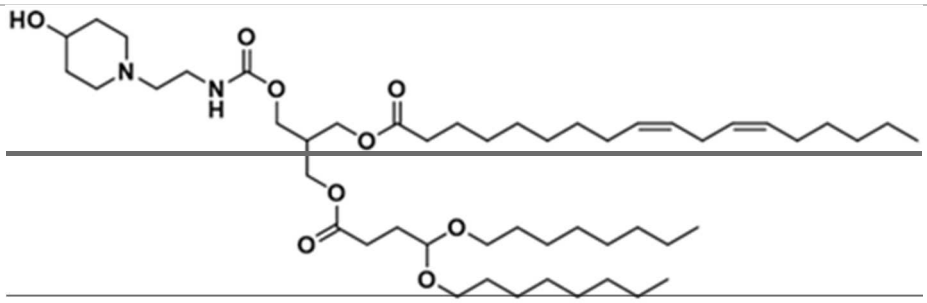


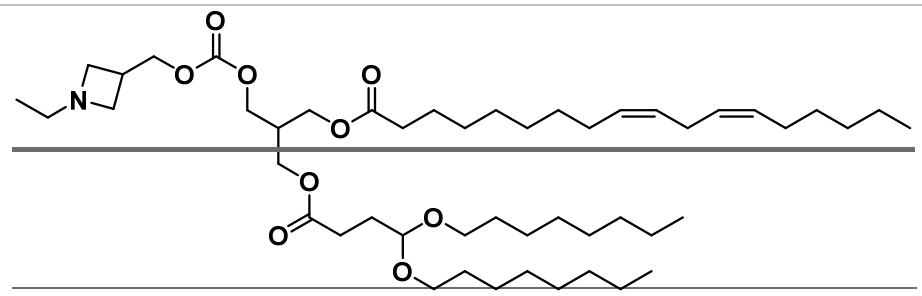


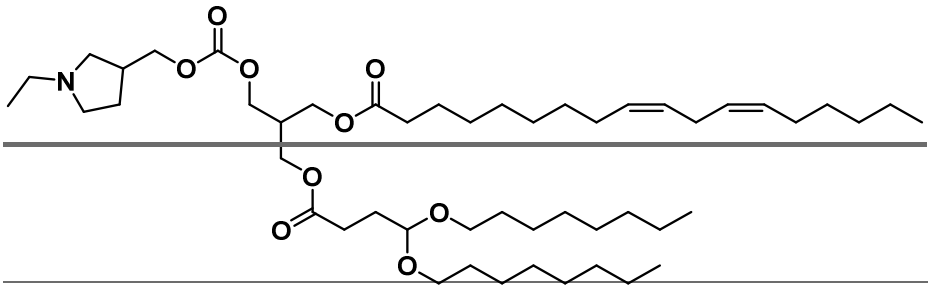


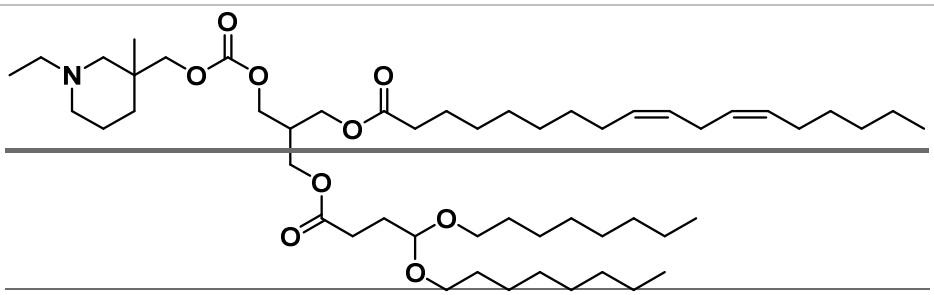


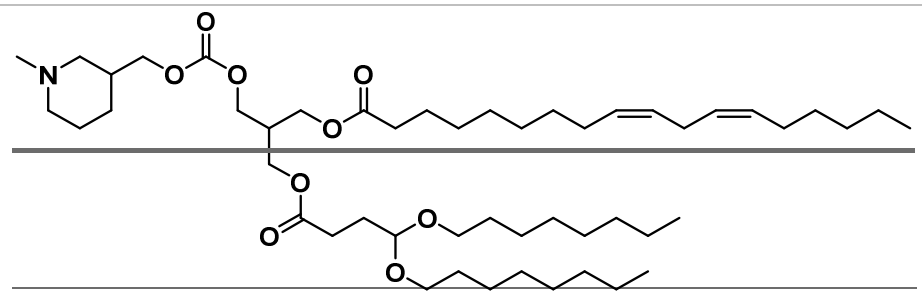


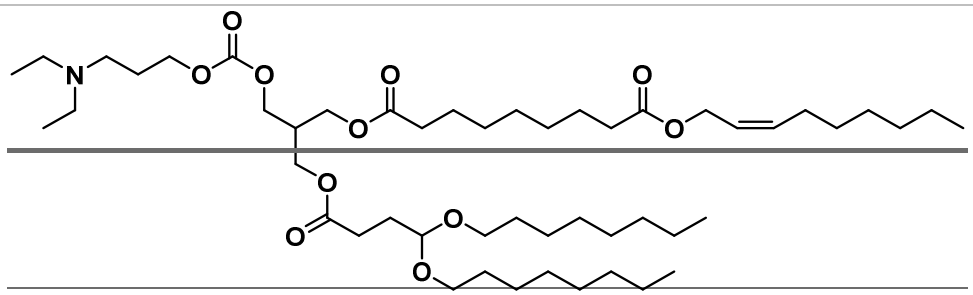


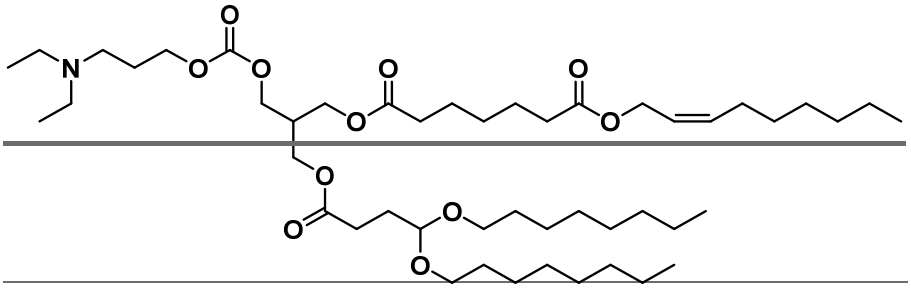


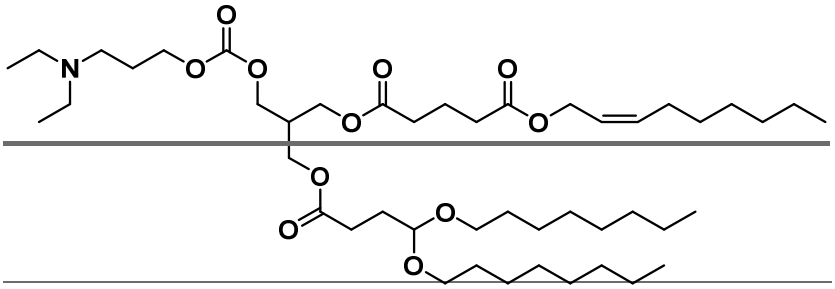


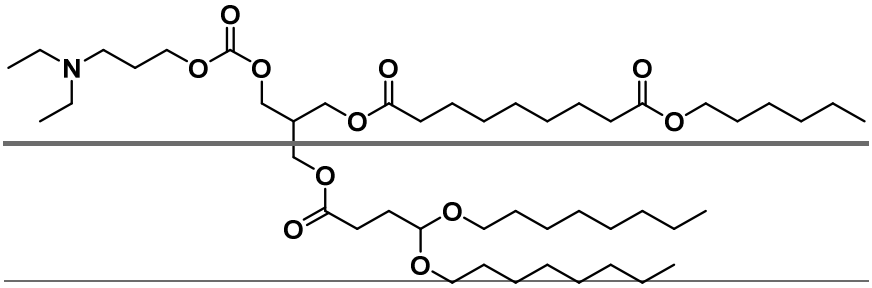


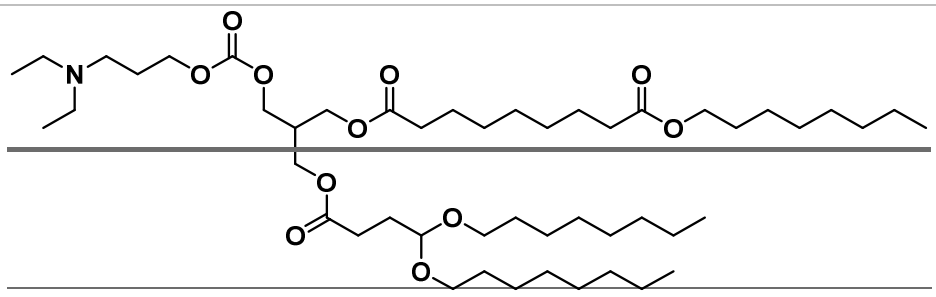


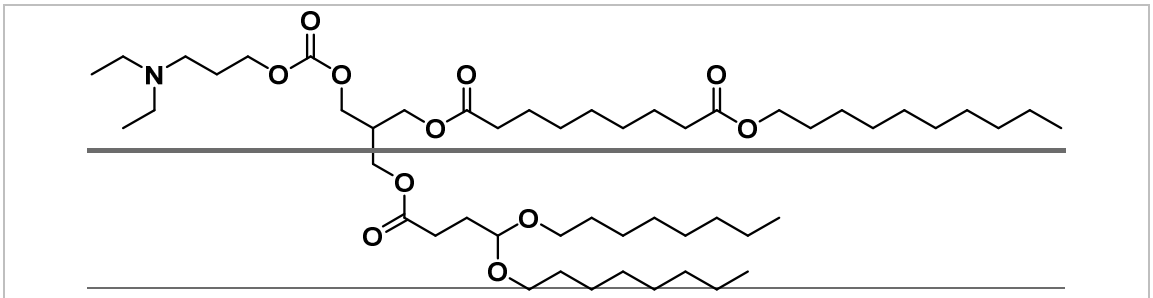


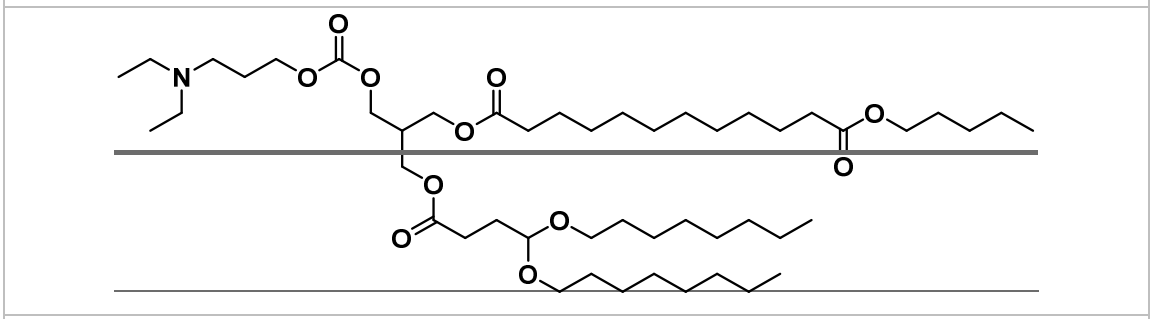


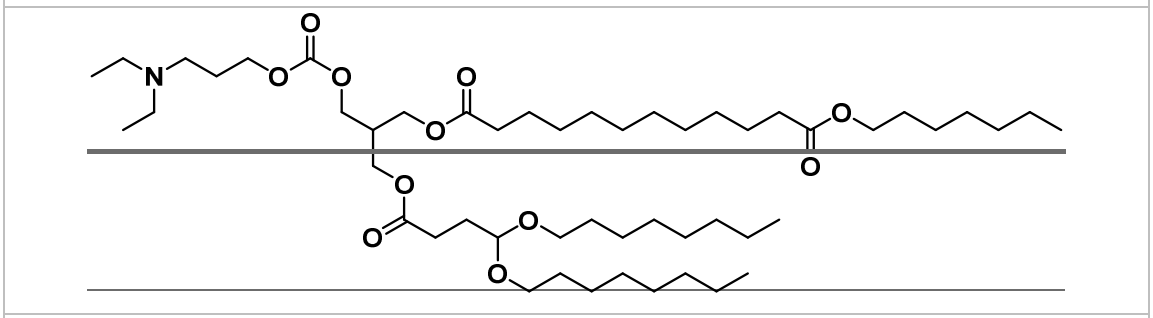


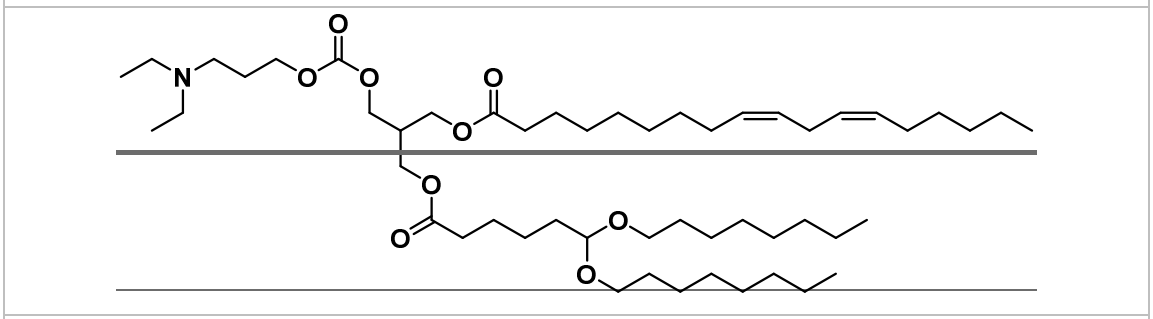


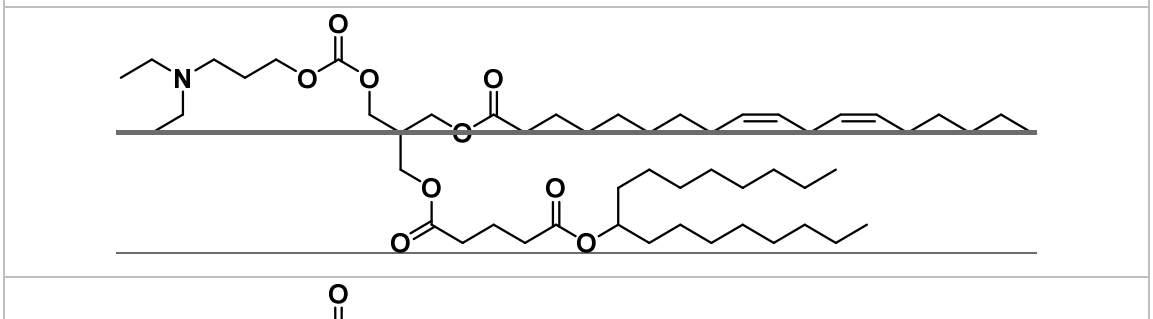


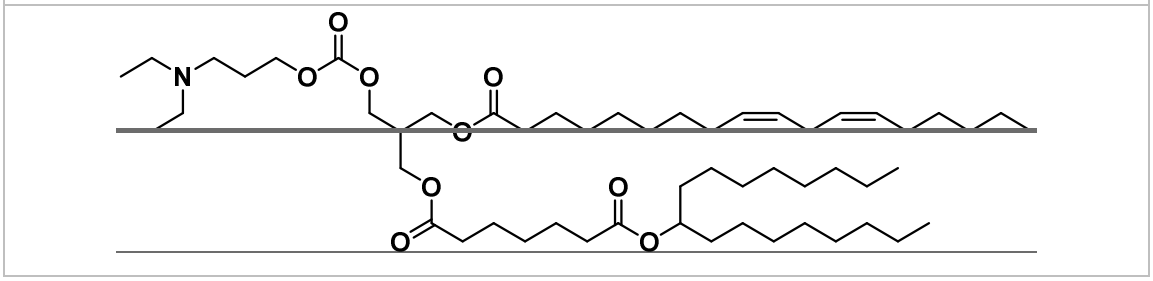


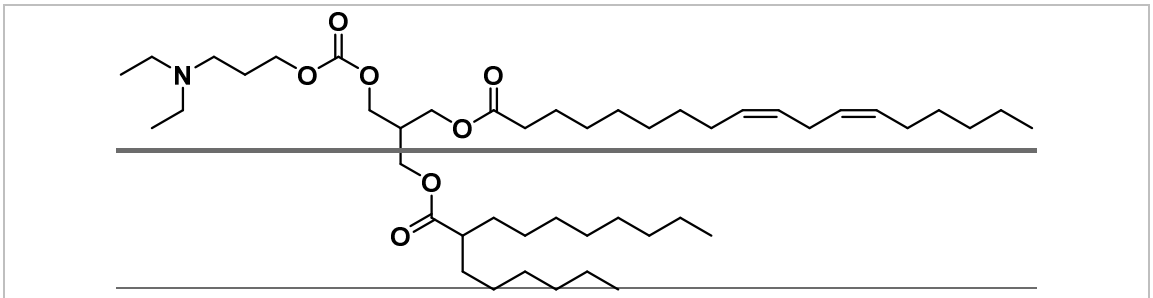


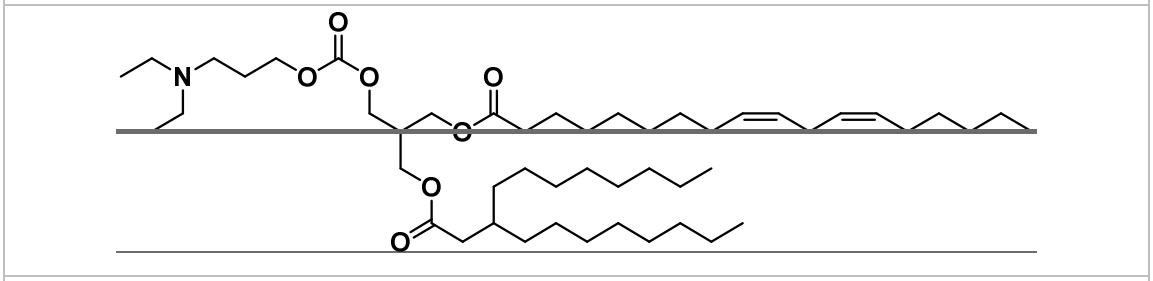


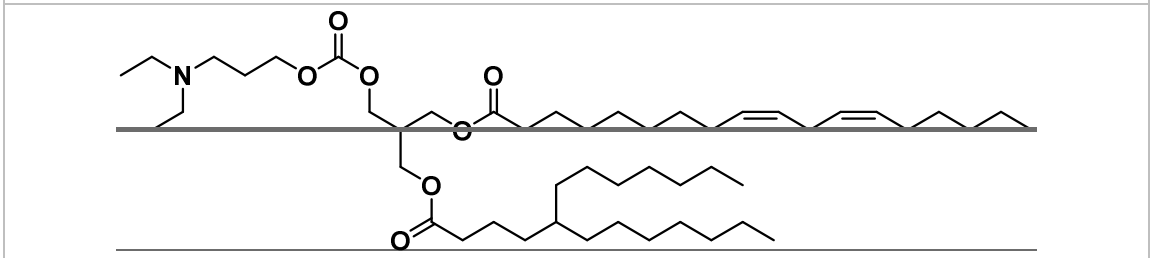


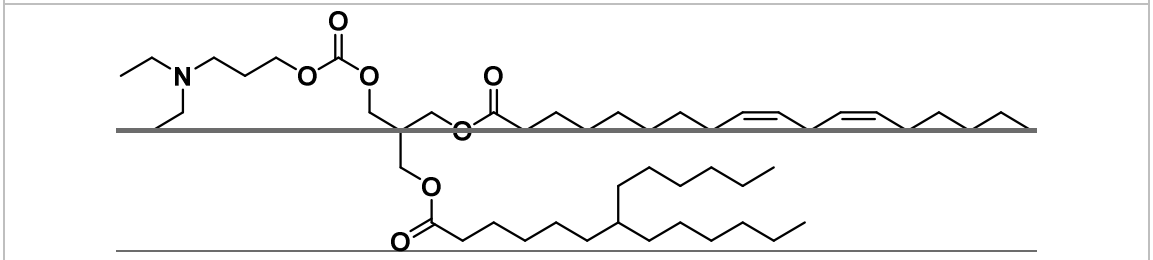


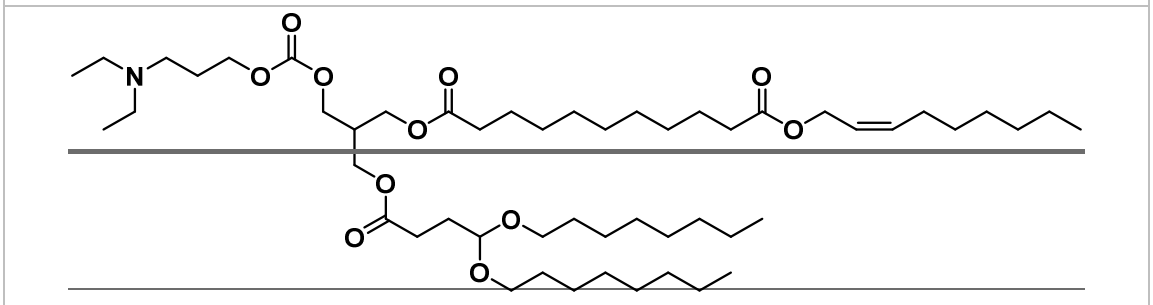


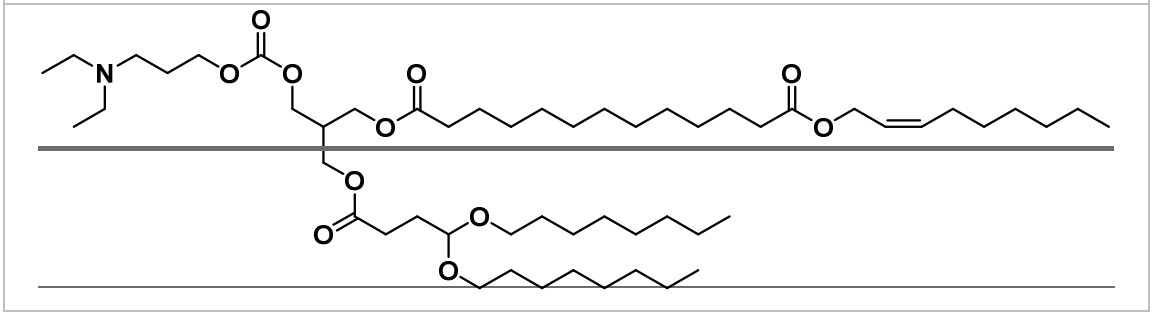


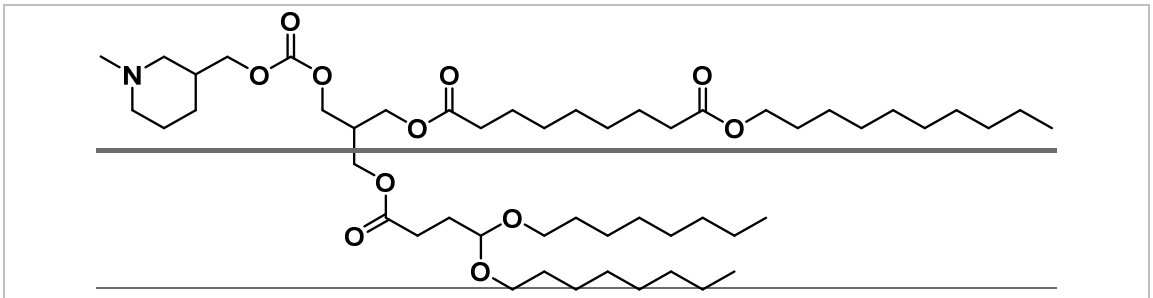


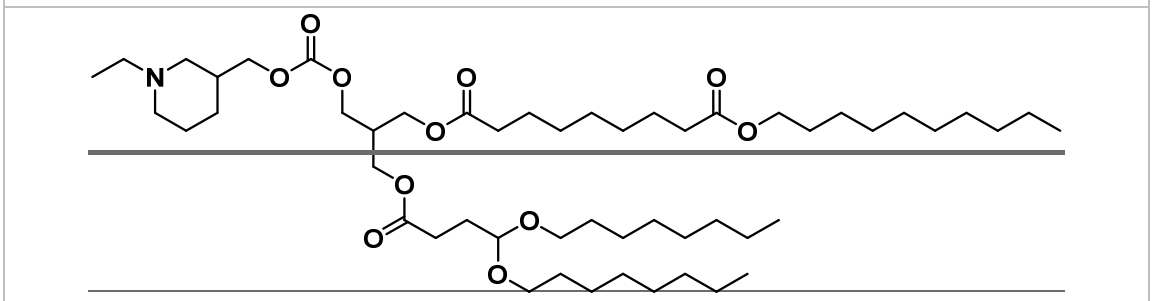


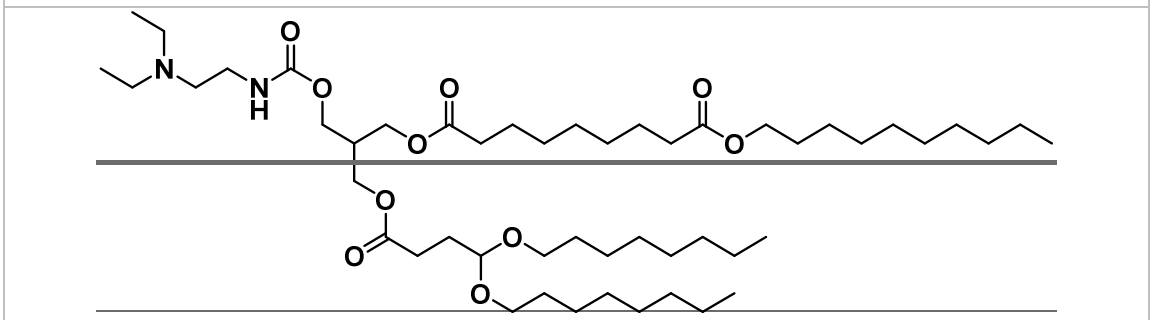


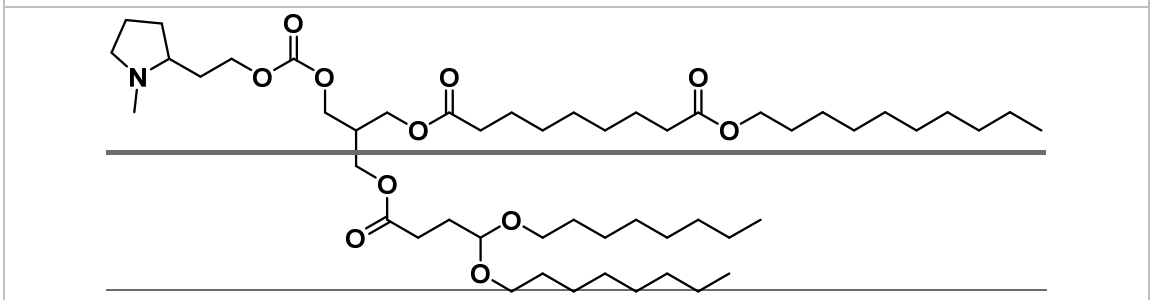


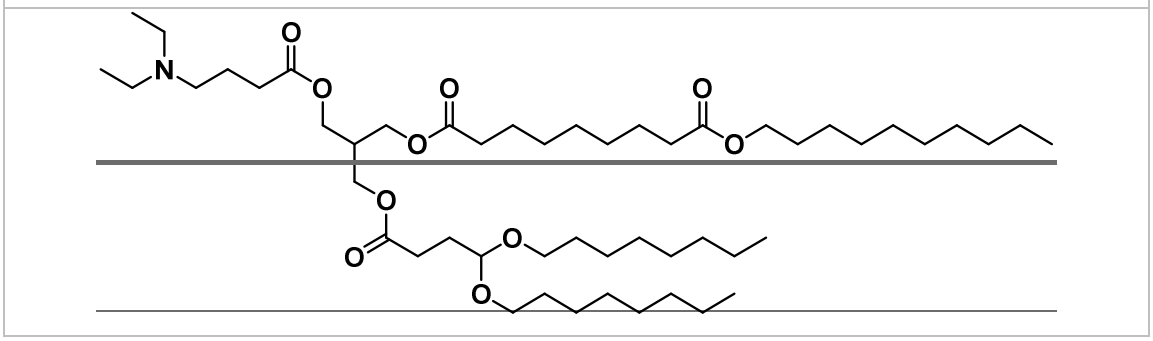


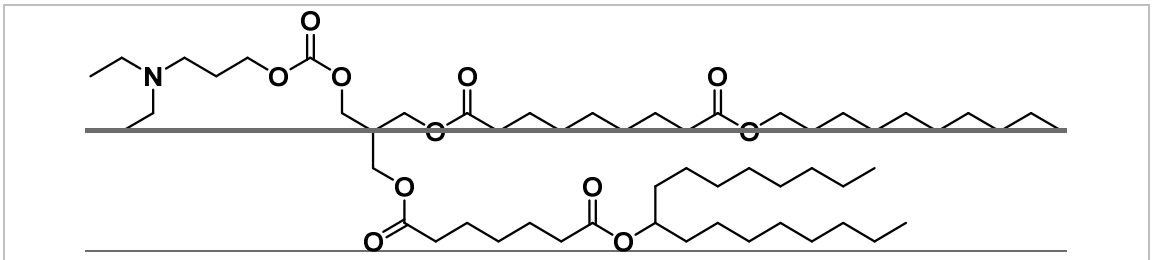


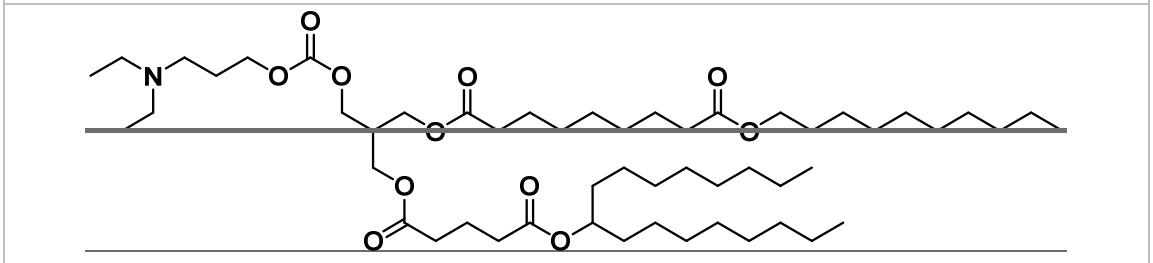


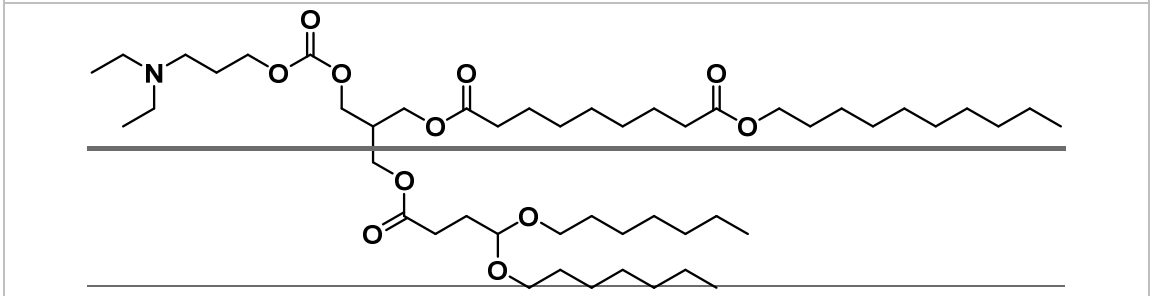


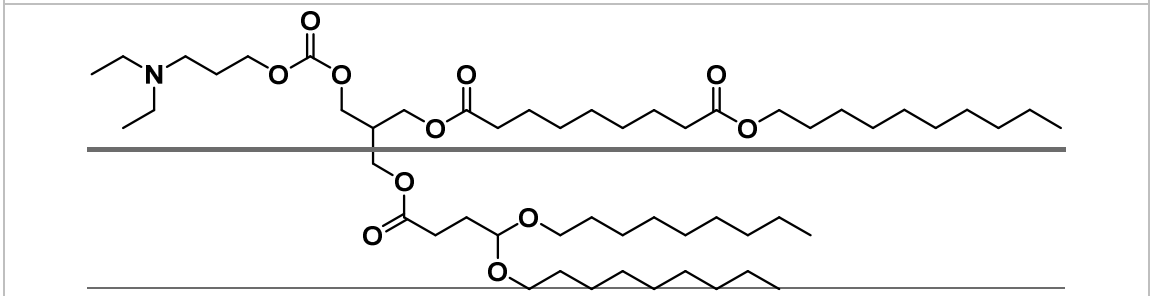


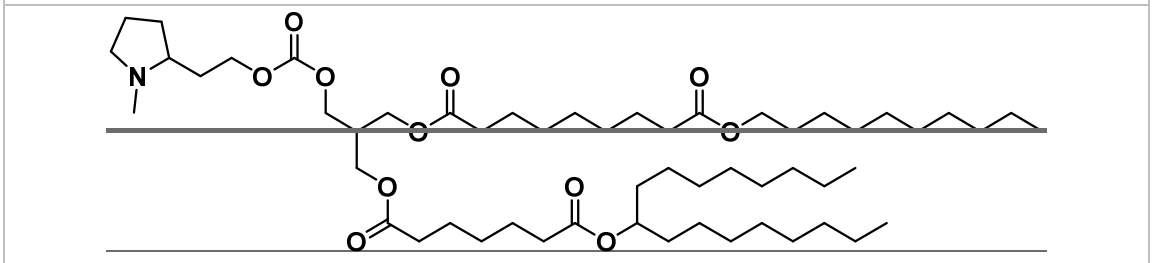


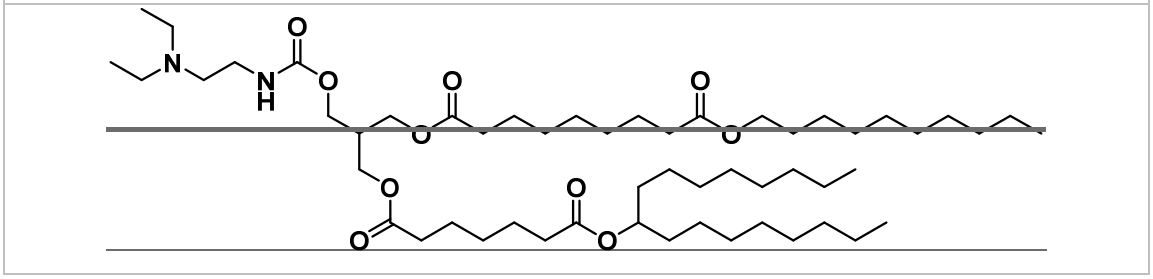


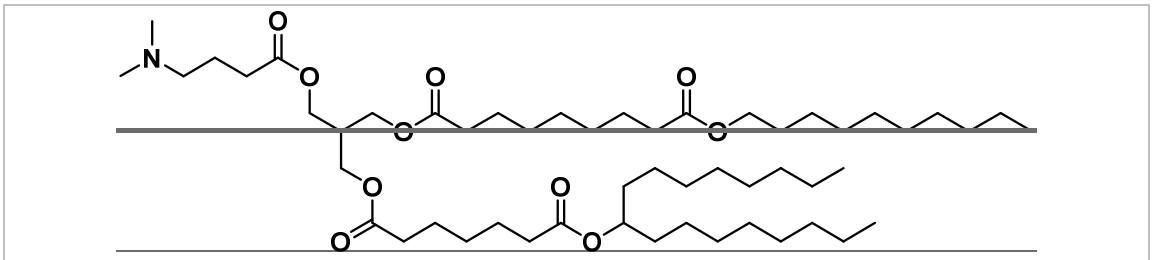


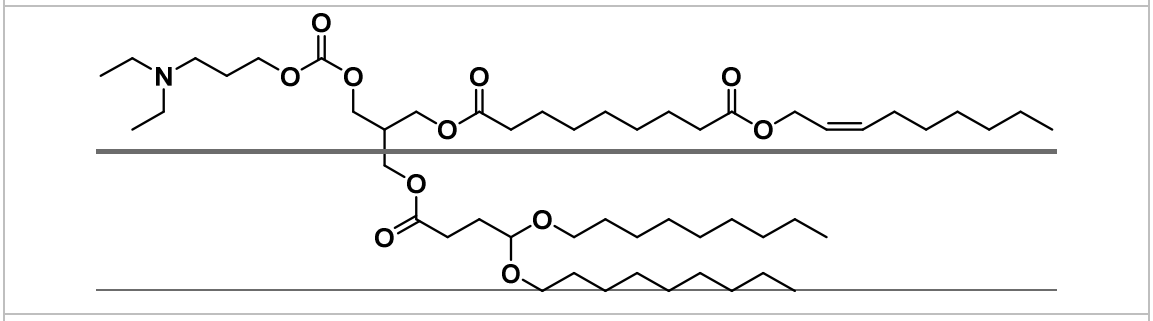


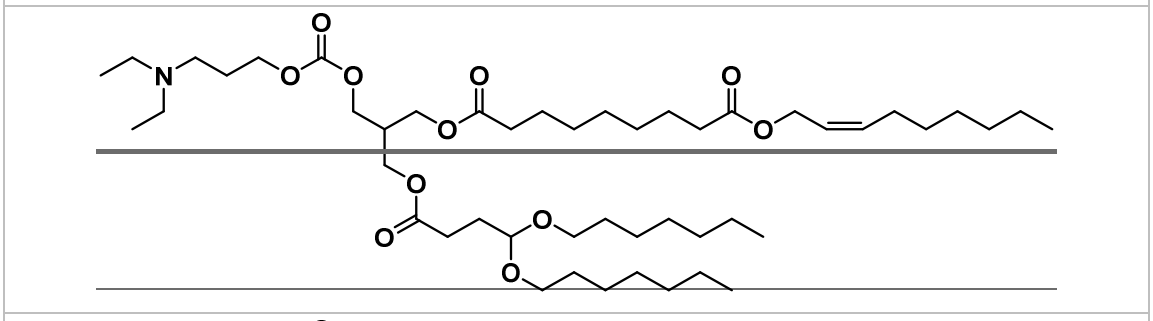


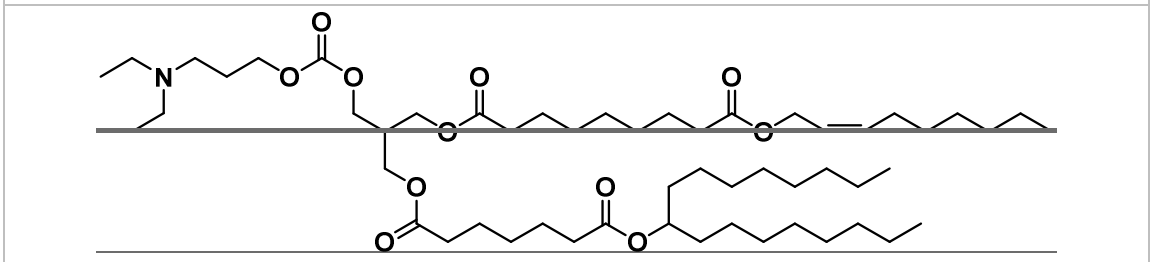


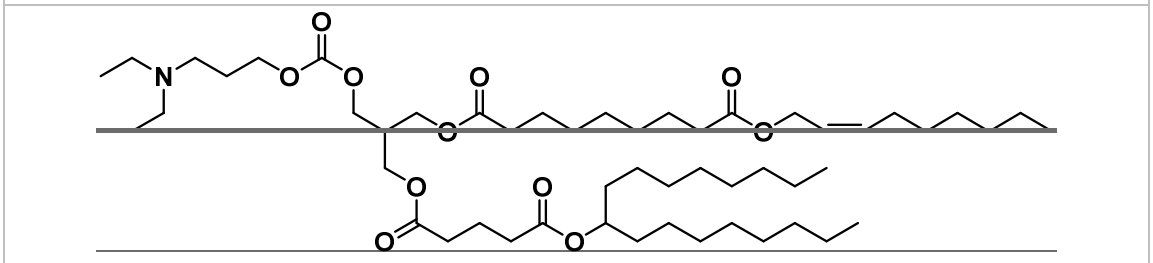


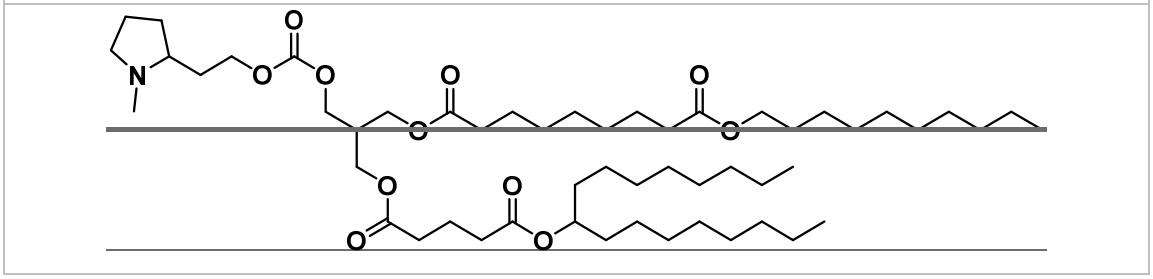


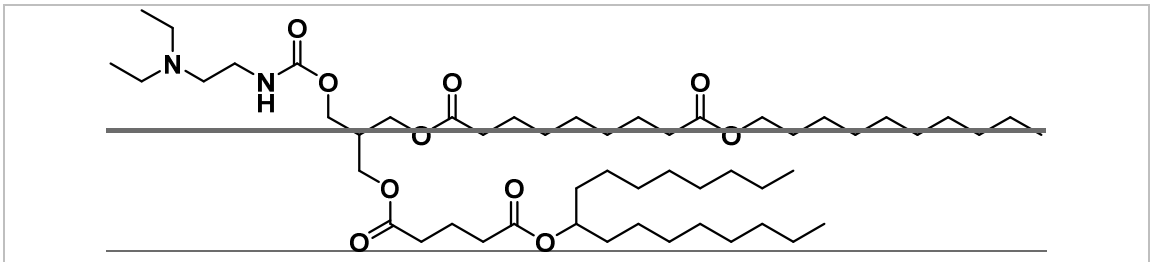


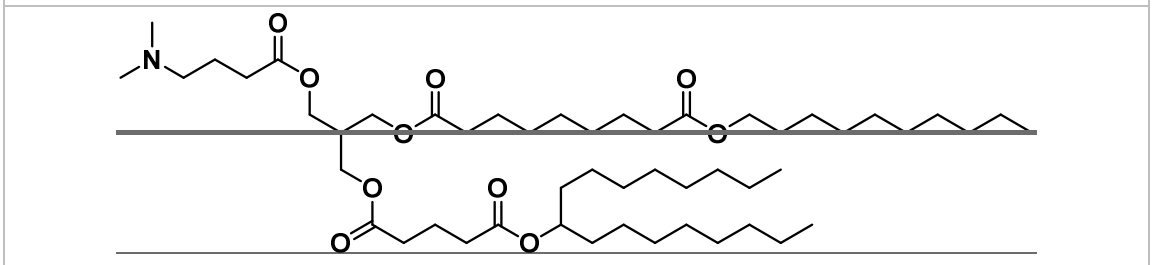


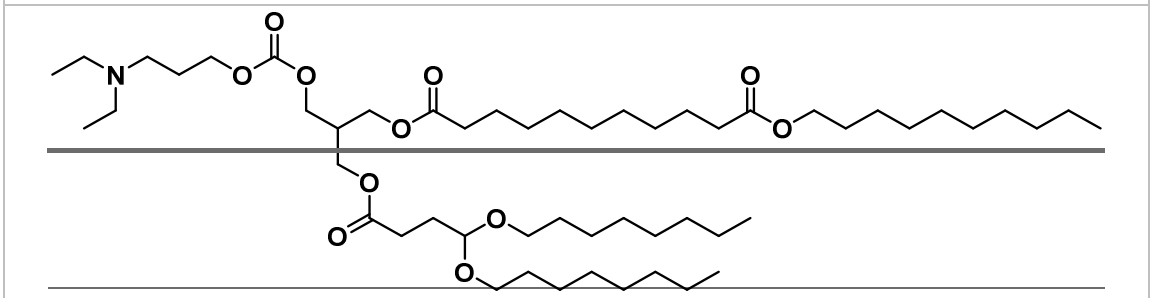


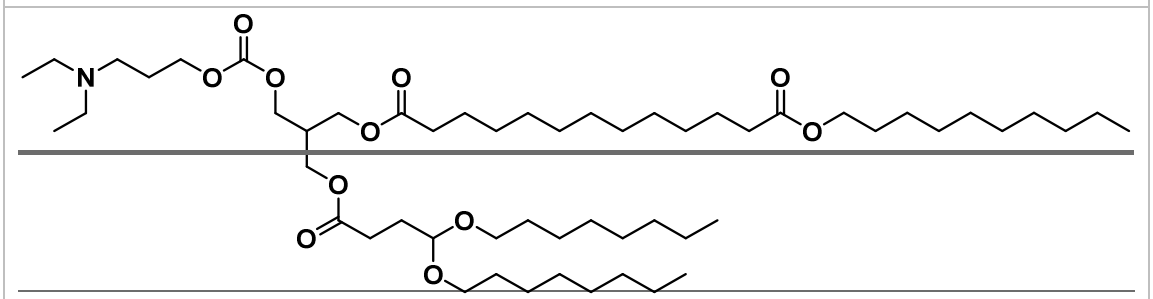


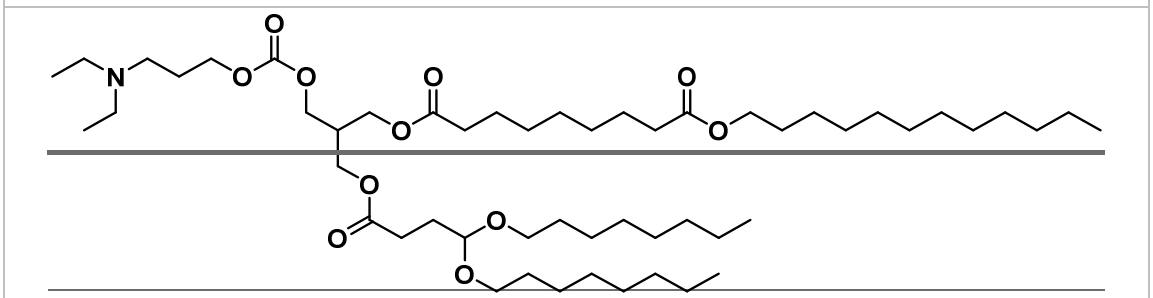


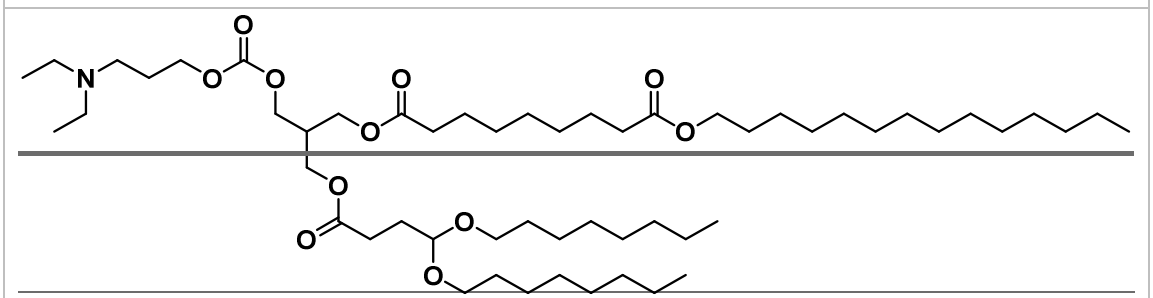


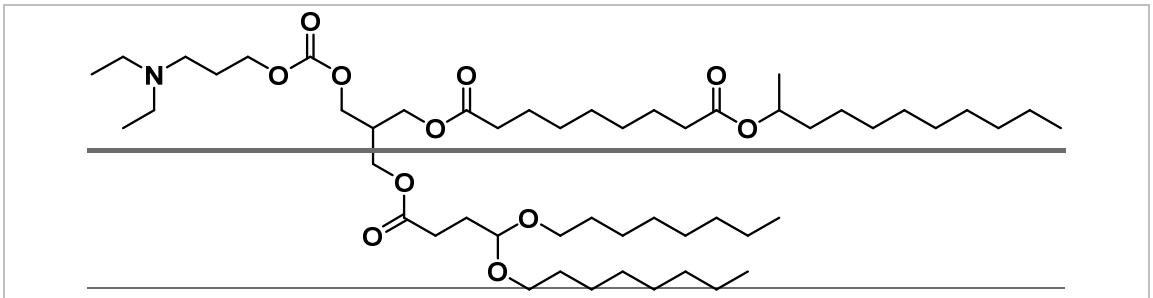


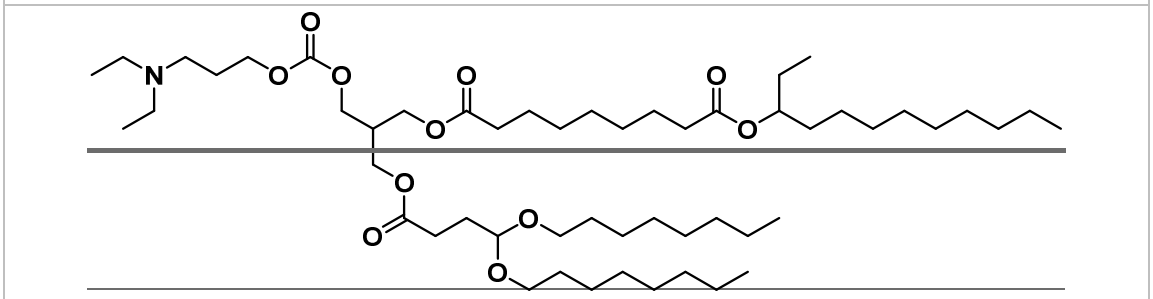


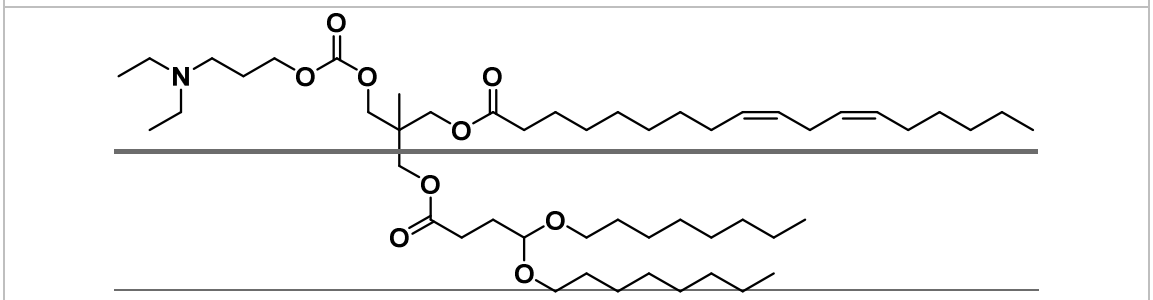


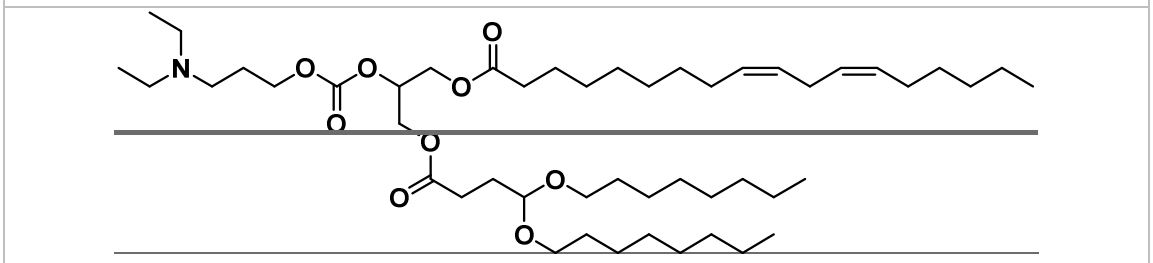


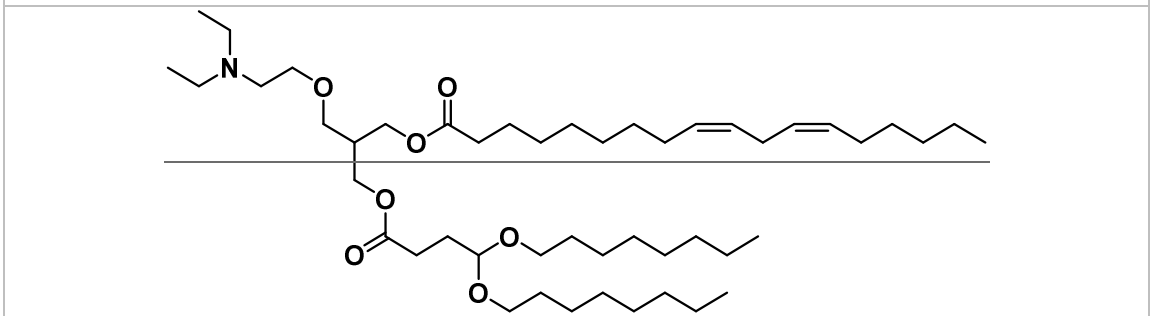


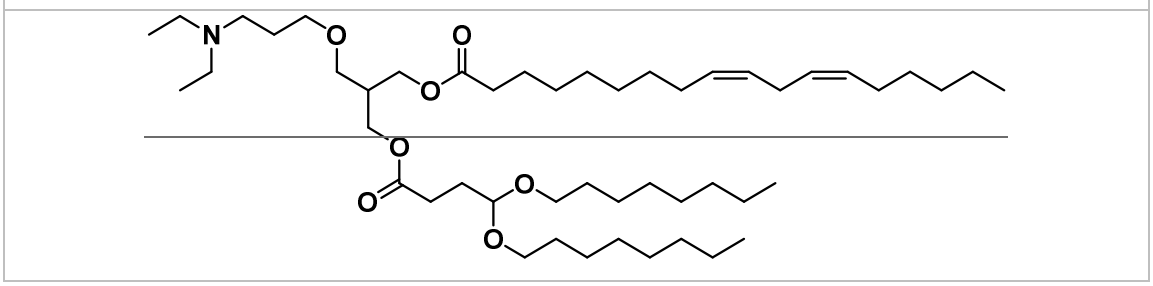


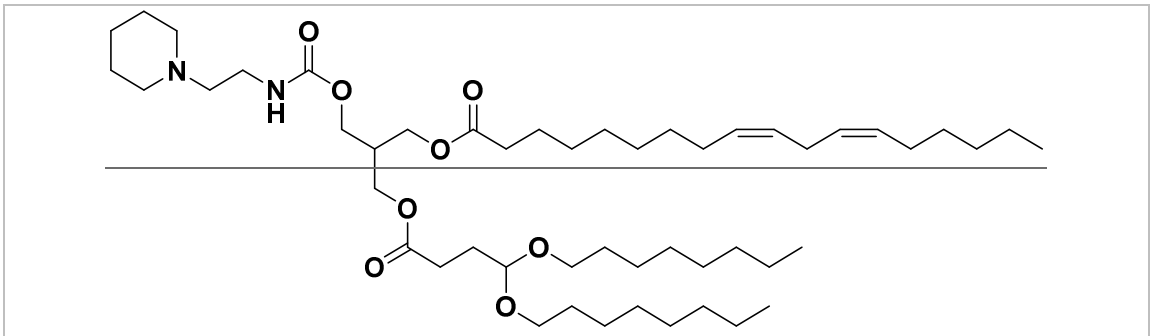


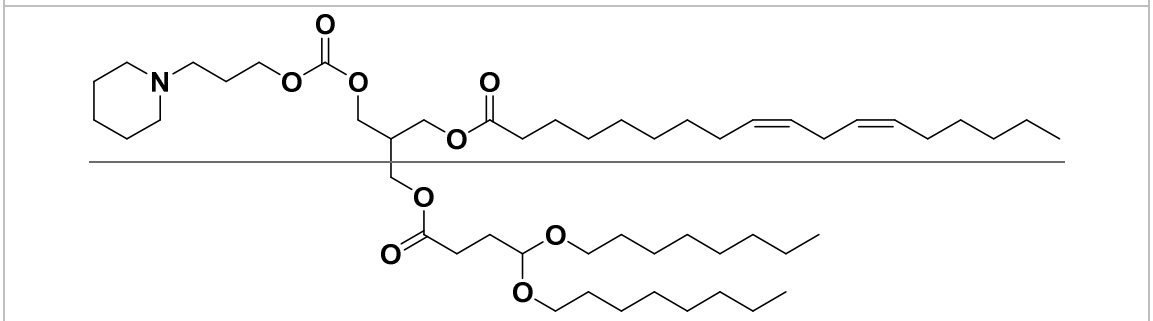


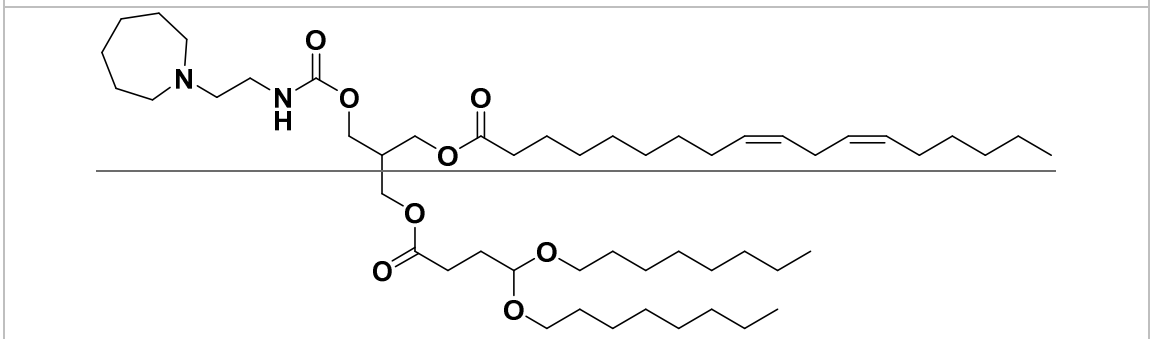


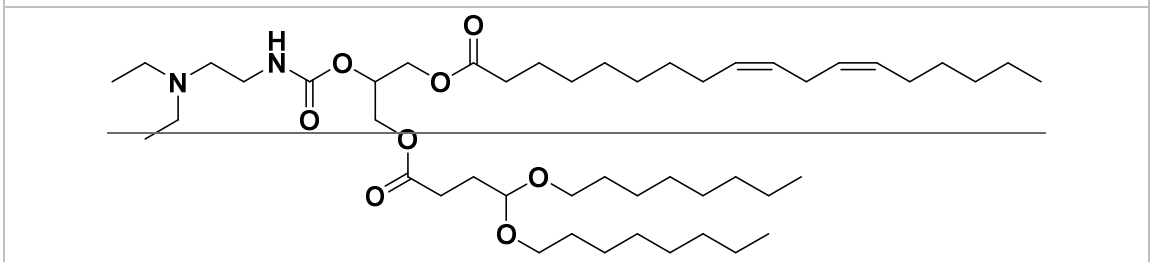


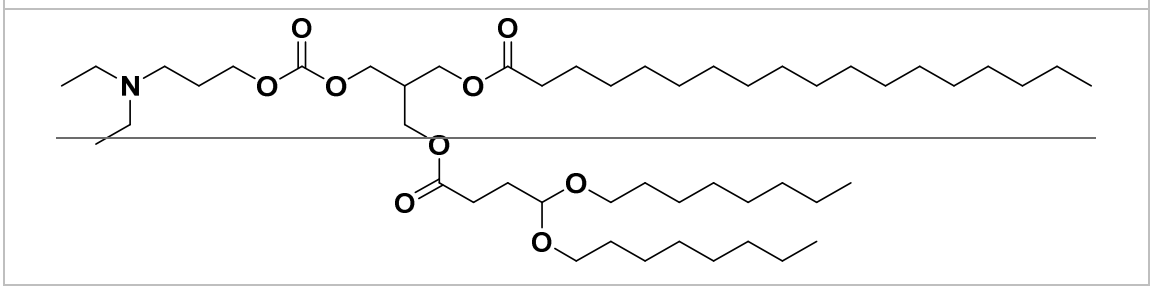


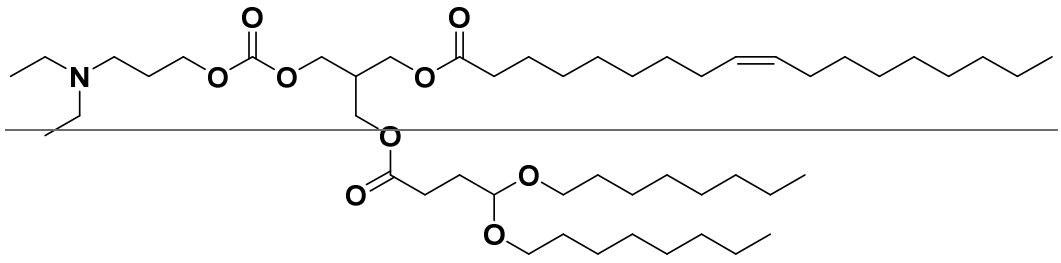


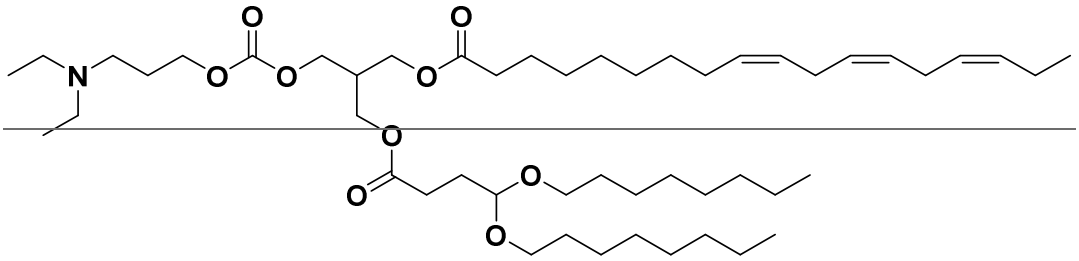












, and

,

or a salt thereof.

30. The compound of any one of claims 1-29, wherein the salt is a pharmaceutically acceptable salt.

31. The compound of any one of the preceding claims, wherein the pKa of the protonated form of the compound is from about 5.1 to about 8.0.

32. The compound of any one of the preceding claims, wherein the pKa of the protonated form of the compound is from about 5.7 to about 7.6.

33. The compound of any one of the preceding claims, wherein the pKa of the protonated form of the compound is from about 6 to about 7.6.

34. A composition comprising a compound of any one of the preceding claims in a lipid component.

35. The composition of claim 34, wherein the composition comprises a lipid component that includes about 50% of the compound of any one of claims 1-31.

36. The composition of claim 34 or 35, wherein the composition is an LNP composition.

37. The composition of any one of claims 34-36, wherein the lipid component comprises a helper lipid and a PEG lipid.

38. The composition of any one of claims 34-37, wherein the lipid component comprises a helper lipid, a PEG lipid, and a neutral lipid.

39. The composition of any one of claims 34-38, further comprising a cryoprotectant.

40. The composition of any one of claims 34-39, further comprising a buffer.

41. The composition of any one of claims 34-40, wherein the composition comprises an aqueous component comprising a biologically active agent.

42. The composition of claim 41, wherein the biologically active agent comprises a polypeptide.

43. The composition of claim 41, wherein the biologically active agent comprises a nucleic acid.

44. The composition of claim 43, wherein the nucleic acid component comprises RNA.

45. The composition of claim 43 or 44, wherein the composition has an N/P ratio of about 3-10.

46. The composition of claim 45, wherein the N/P ratio is about 6 ± 1.

47. The composition of claim 45, wherein the N/P ratio is about 6 ± 0.5.

48. The composition of claim 45, wherein the N/P ratio is about 6.

49. The composition of any one of claims 34-48, comprising an RNA component, wherein the RNA component comprises an mRNA.

50. The composition of claim 49, wherein the RNA component comprises a sequence encoding RNA-guided DNA-binding agent, such as a Cas nuclease mRNA.

51. The composition of claim 49 or 50, wherein the RNA component comprises a Class 2 Cas nuclease mRNA.

52. The composition of any one of claims 49-51, wherein the RNA component comprises a Cas9 nuclease mRNA.

53. The composition of any one of claims 49-52, wherein the RNA component comprises a modified RNA.

~~54. The composition of any one of claims 49-53, wherein the RNA component comprises a gRNA nucleic acid.~~

~~55. The composition of claim 54, wherein the gRNA nucleic acid is a gRNA.~~

~~56. The composition of any one of claims 49-55, wherein the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA.~~

~~57. The composition of any one of claims 54-56, wherein the gRNA nucleic acid is or encodes a dual-guide RNA (dgRNA).~~

~~58. The composition of any one of claims 54-56, wherein the gRNA nucleic acid is or encodes a single-guide RNA (sgRNA).~~

~~59. The composition of any one of claims 54-58, wherein the gRNA is a modified gRNA.~~

~~60. The composition of claim 59, wherein the modified gRNA comprises a modification at one or more of the first five nucleotides at a 5' end.~~

~~61. The composition of claims 59 or 60, wherein the modified gRNA comprises a modification at one or more of the last five nucleotides at a 3' end.~~

~~62. The composition of any one of claims 41-61, further comprising at least one template nucleic acid.~~

~~63. A method of delivering a biologically active agent to a cell, comprising contacting the cell with a composition of any one of claims 34-62.~~

~~64. The method of claim 63, wherein the cell is a liver cell, such as a hepatocyte.~~

~~65. A method of gene editing, comprising contacting a cell with a composition of any one of claims 34-62.~~

~~66. A method of cleaving a DNA, comprising contacting a cell with a composition of any one of claims 34-62.~~

~~67. The method of claim 66, wherein the contacting step results in a single stranded DNA nick.~~

~~68. The method of claim 66, wherein the contacting step results in a double-stranded DNA break.~~

~~69. The method of any one of claims 63-67, wherein the composition comprises a Class 2 Cas mRNA and a gRNA nucleic acid.~~

~~70. The method of any one of claims 63-69, further comprising introducing at least one template nucleic acid into the cell.~~

~~71. The method of claim 70, comprising contacting the cell with a composition comprising a template nucleic acid.~~

~~72. The method of any one of claims 63-71, wherein the method comprises administering the composition to an animal.~~

~~73. The method of any one of claims 63-71, wherein the method comprises administering the composition to a human.~~

~~74. The method of any one of claims 63-71, wherein the method comprises administering the composition to a cell.~~

~~75. The method of claim 74, wherein the cell is a eukaryotic cell.~~

~~76. The method of any one of claims 63-75, wherein the method comprises administering the mRNA formulated in a first LNP composition and a second LNP composition comprising one or more of an mRNA, a gRNA, a gRNA nucleic acid, and a template nucleic acid.~~

~~77. The method of claim 76, wherein the first and second LNP compositions are administered simultaneously.~~

~~78. The method of claim 76, wherein the first and second LNP compositions are administered sequentially.~~

~~79. The method of claim 76, wherein the method comprises administering the mRNA and the gRNA nucleic acid formulated in a single LNP composition.~~

~~80. The method of any one of claims 63-79, wherein the gene editing results in a gene knockout.~~

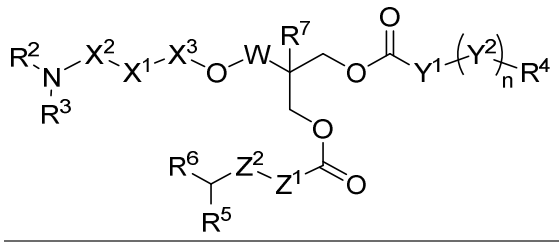
~~81. The method of any one of claims 63-79, wherein the gene editing results in a gene correction.~~

~~82. The method of any one of claims 63-81, wherein the method comprises administering the composition to a liver cell.~~

~~83. The method of any one of claims 63-82, wherein the method comprises administering the composition to a hepatocyte.~~

~~84. The method of any one of claims 63-83, wherein the method comprises administering the composition to a liver.~~

1. A compound of Formula II



(II),

wherein

X^2 is C_{2-5} alkylene,

X^3 is C(=O) or a direct bond,

Y^1 is C_{2-12} alkylene,

n is 0 to 3,

R^4 is C_{1-15} alkyl,

R^5 is C_{5-9} alkyl or C_{6-10} alkoxy,

R^6 is C_{5-9} alkyl or C_{6-10} alkoxy,

W is methylene or a direct bond,

R^7 is H or Me, and

(i) X^1 is O, NR^1, or a direct bond,

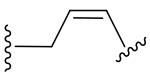
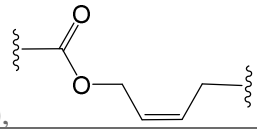
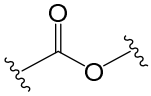
R^1 is H or Me,

R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-, 6-, or 7-membered ring, or

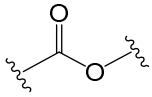
R^3 is C_{1-3} alkyl and R^2 is C_{1-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or

X^1 is NR^1, and R^1 and R^2 taken together with the nitrogen atoms to which they are attached form a 5- or 6-membered ring, and

Y^2 is selected from  (in either orientation),  (in either orientation), and  (in either orientation),

Z^1 is C_{1-6} alkylene, and

Z^2 is  (in either orientation), or

(ii) X^1 is O, NR^1, or a direct bond,

R^1 is H or Me,

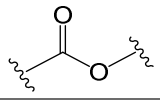
R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-, 6-, or 7-membered ring, or

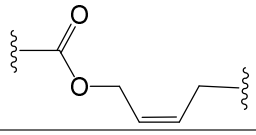
R^3 is C_{1-3} alkyl and R^2 is C_{1-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or

X^1 is NR^1, and R^1 and R^2 taken together with the nitrogen atoms to which they are attached form a 5- or 6-membered ring, and

Z^1 is C_{1-6} alkylene or a direct bond,

Z^2 is  (in either orientation) or absent, provided that if Z^1 is a direct bond, Z^2 is absent, and

Y^2 is  (in either orientation), or

(iii) X^1 is NR^1,

R^1 is H or Me,

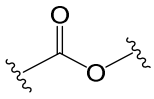
R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-, 6-, or 7-membered ring, or

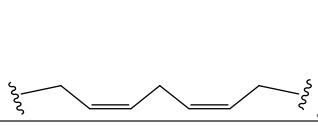
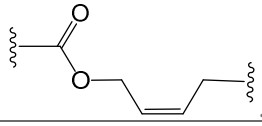
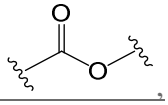
R^3 is C_{2-3} alkyl and R^2 is C_{2-3} alkyl, or

R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or

X^1 is NR^1, and R^1 and R^2 taken together with the nitrogen atoms to which they are attached form a 5- or 6-membered ring,

Z^1 is C_{1-6} alkylene or a direct bond,

Z^2 is  (in either orientation) or absent, provided that if Z^1 is a direct bond, Z^2 is absent, and

Y^2 is selected from , , and ,

or a salt thereof.

2. The compound of claim 1, wherein X^2 is linear C_2 alkylene, linear C_3 alkylene, or linear C_4 alkylene.

3. The compound of any one of the preceding claims, wherein R^3 is C_1 alkyl or C_2 alkyl.

4. The compound of any one of the preceding claims, wherein R^2 is C_1 alkyl or C_2 alkyl.

5. The compound of claims 1-3, wherein R^2 taken together with the nitrogen atom and 1-2 carbon atoms of X^2 form a 5-membered ring or a 6-membered ring.

6. The compound of claim 1 or 2, wherein R^2 and R^3 taken together with the nitrogen atom form a 5-membered ring.

7. The compound of any one of the preceding claims, wherein X^1 is NH.

8. The compound of claims 1-6, wherein X^1 is a direct bond.

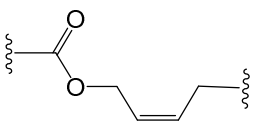
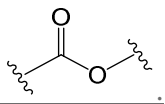
9. The compound of any one of the preceding claims, wherein Y^1 is linear C_{3-10} alkylene, linear C_{4-8} alkylene, or linear C_{5-7} alkylene.

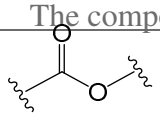
10. The compound of any one of the preceding claims, wherein R^4 is linear C_{4-14} alkyl or linear C_{6-12} alkyl.

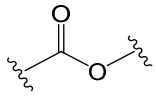
11. The compound of any one of the preceding claims, wherein Z^1 is linear C_{2-4} alkylene.

12. The compound of any one of the preceding claims, wherein R^5 and R^6 are each independently linear C_{5-9} alkyl, independently linear C_{6-8} alkyl, or independently linear C_{7-9} alkoxy.

13. The compound of any one of the preceding claims, wherein Y^2 is

 or .

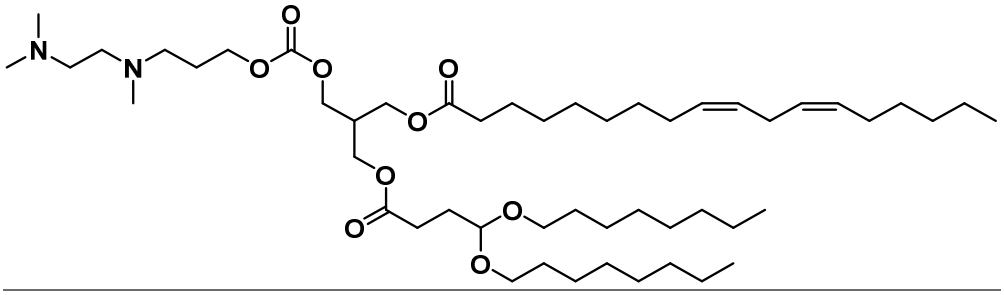
14. The compound of any one of the preceding claims, wherein Y^1 is linear C_7 alkylene, Y^2 is , n = 1, and R^4 is linear C_{10} alkyl.

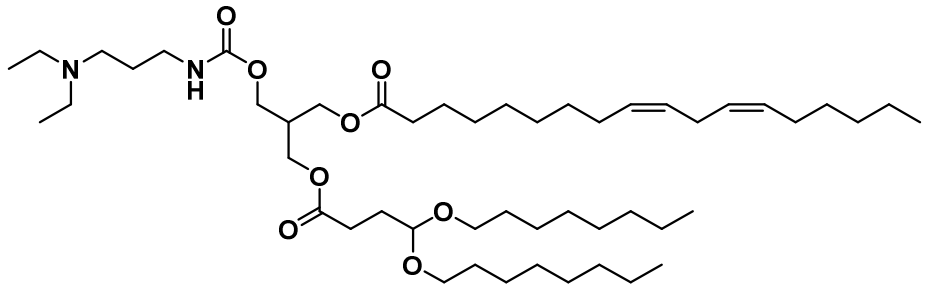
15. The compound of any one of the preceding claims, wherein Z^2 is .

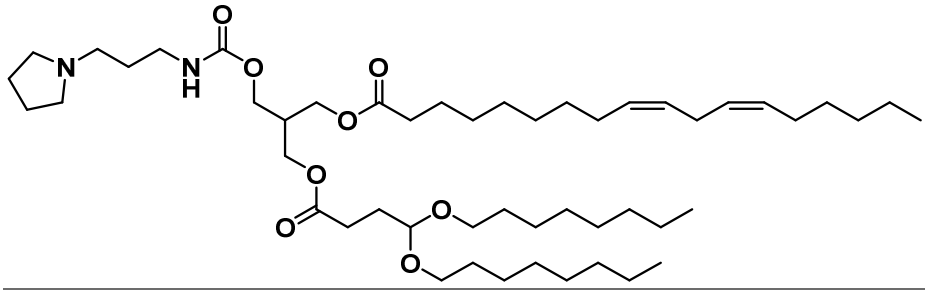
16. The compound of any one of the preceding claims, wherein Z^1, Z^2, and R^5 are selected to form a linear chain of 6-18 atoms, including the carbon and oxygen atoms of the ester(s) and the acetal.

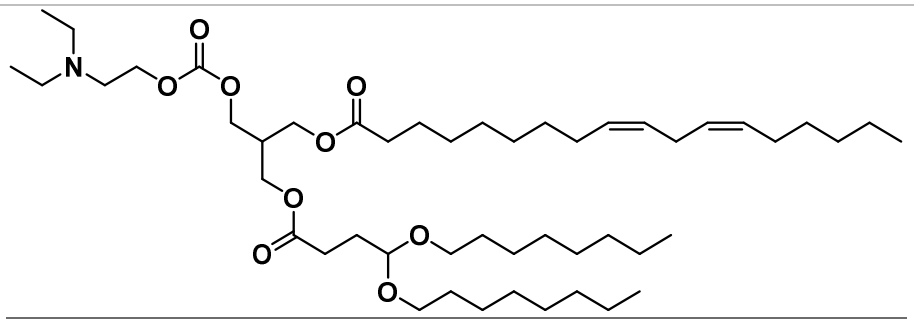
17. The compound of any one of the preceding claims, wherein Y^1, Y^2, and R^4 are selected to form a linear chain of 14-24 atoms, including the carbon and oxygen atoms of the ester(s), if present.

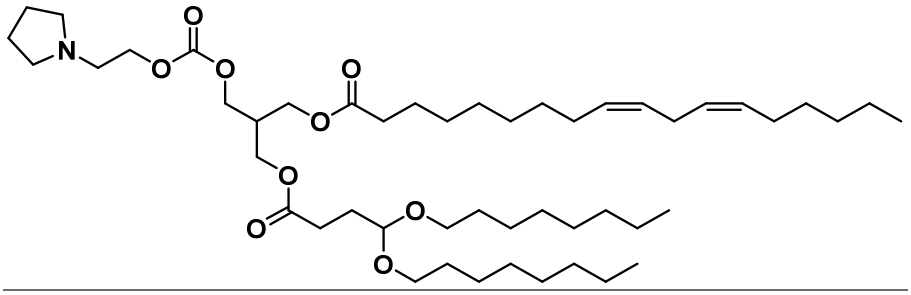
18. A compound selected from:

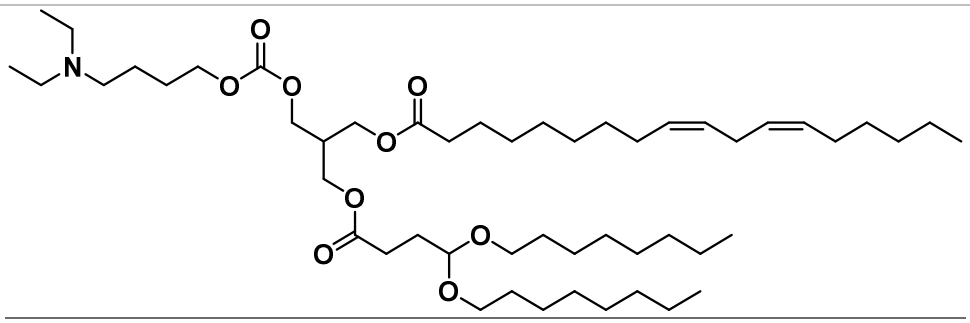


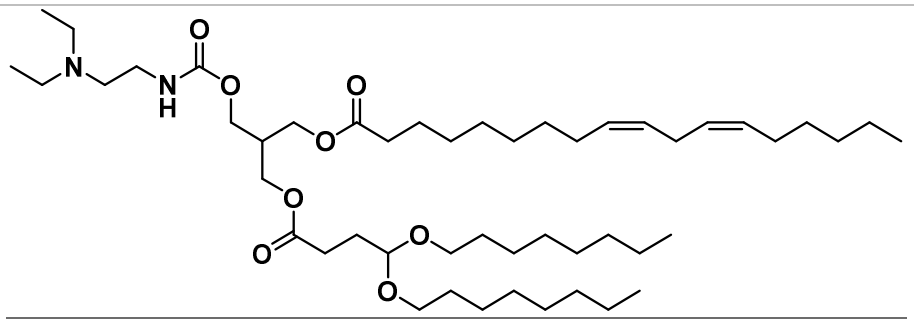


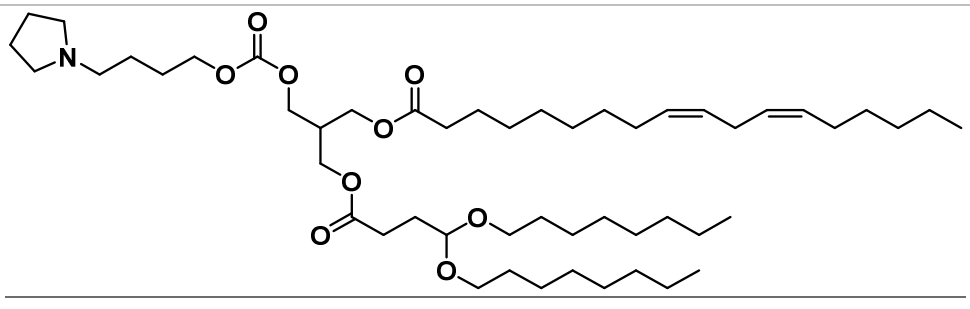


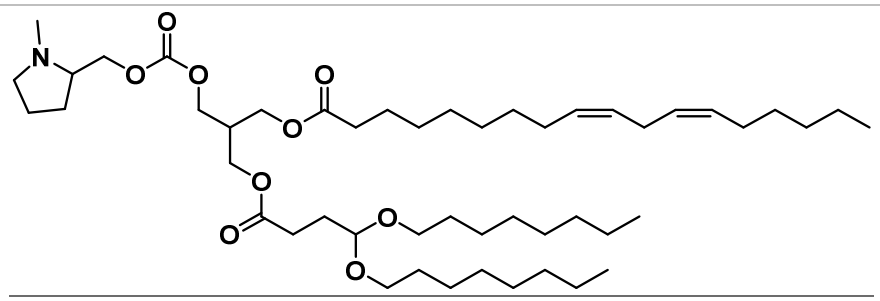


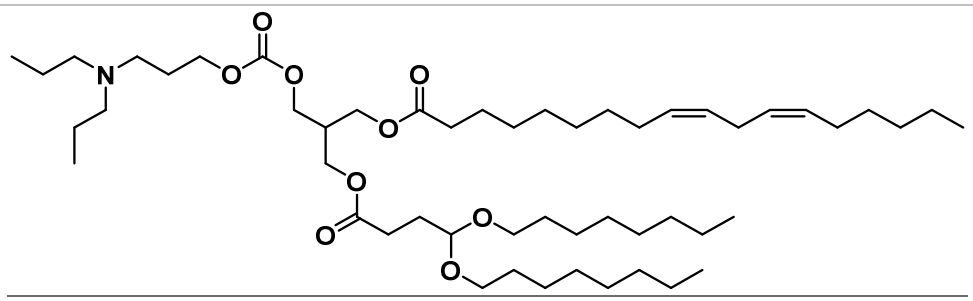


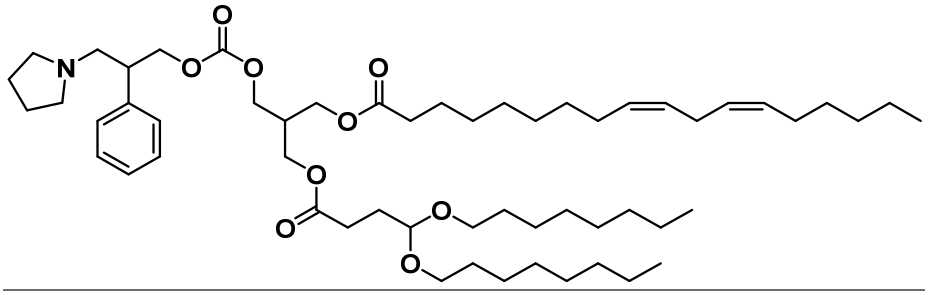


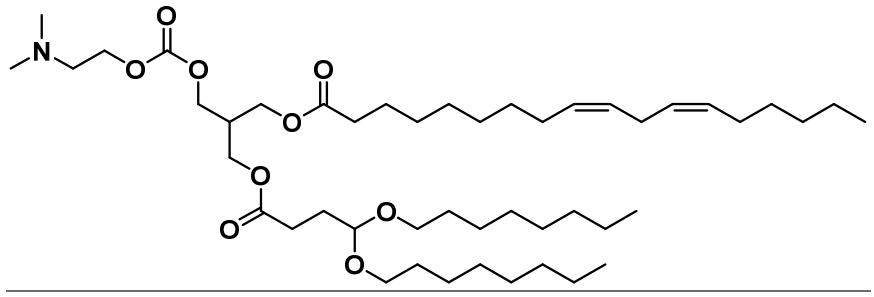


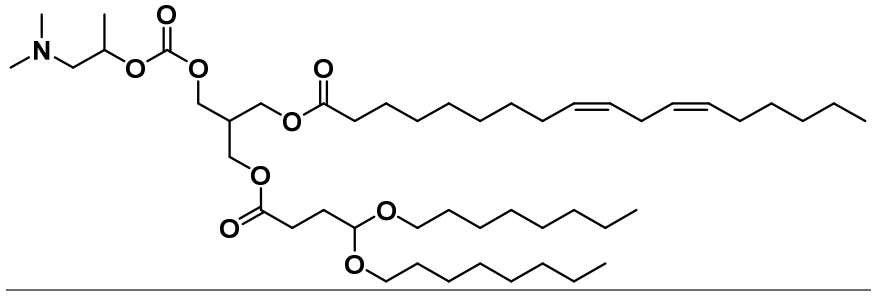


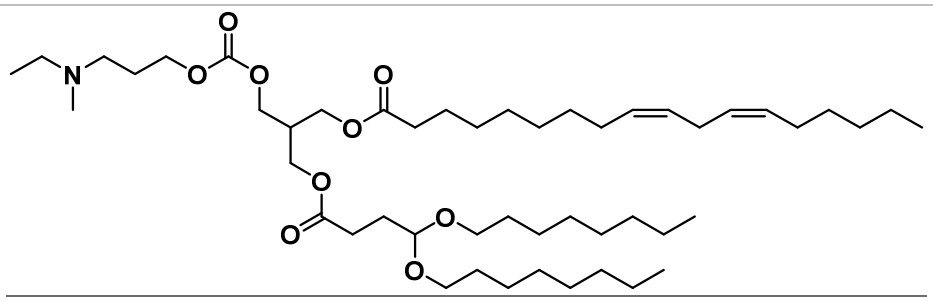


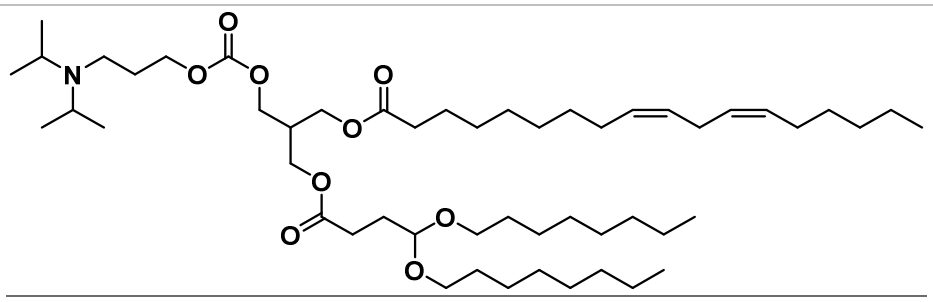


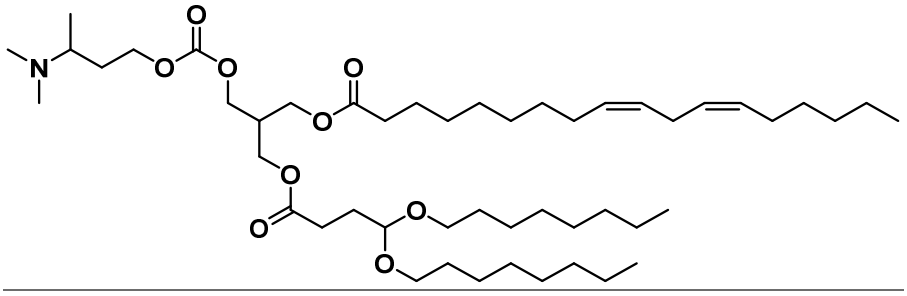


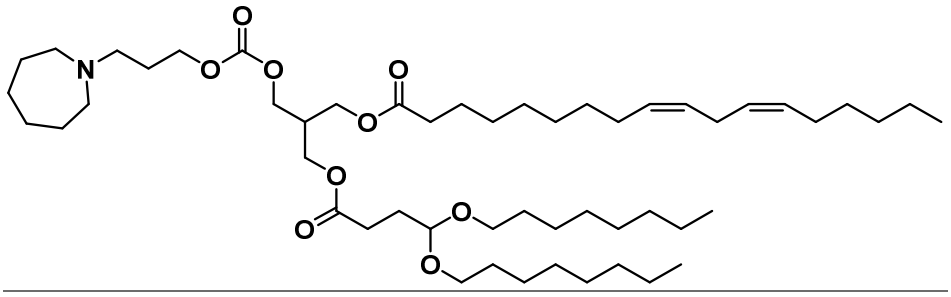


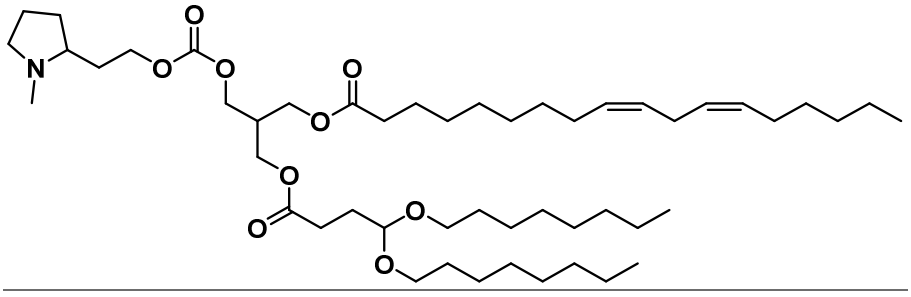


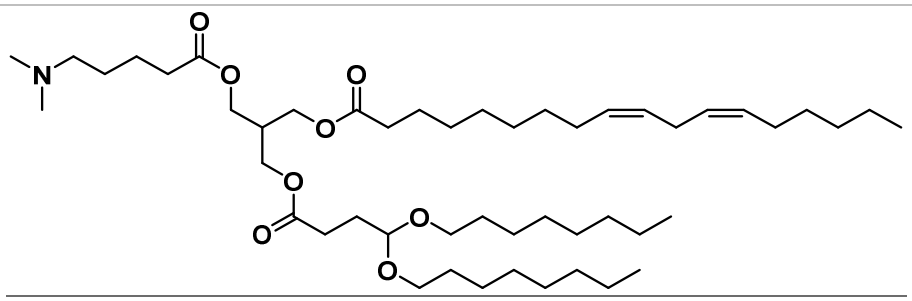


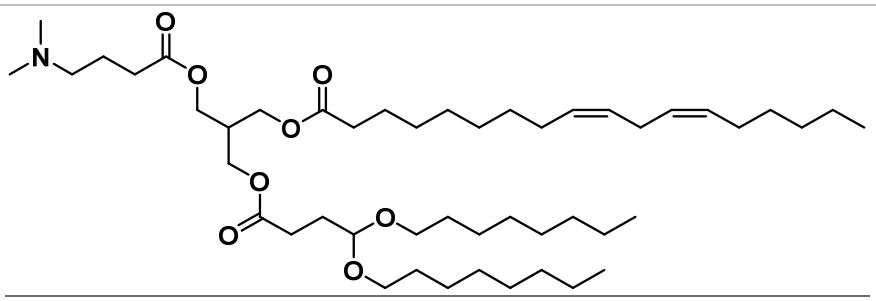


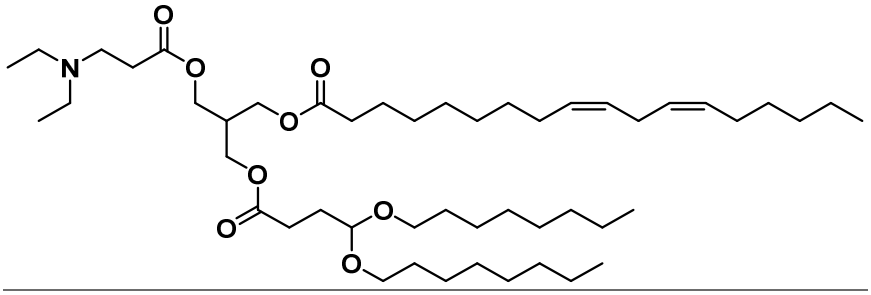


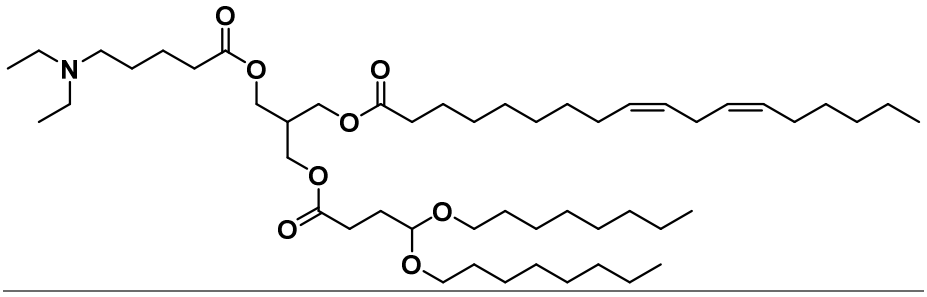


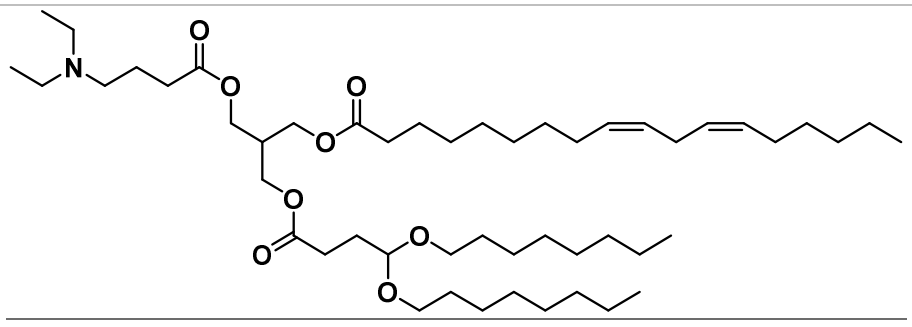


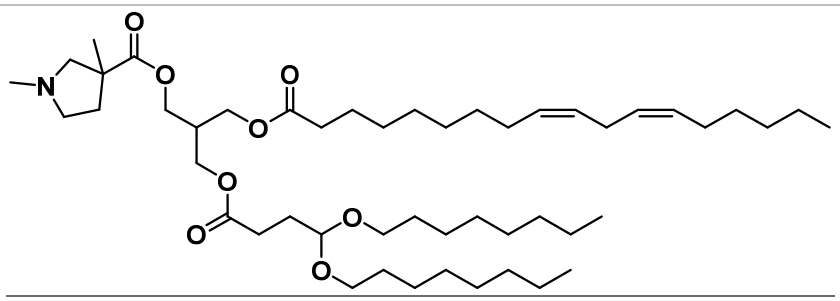


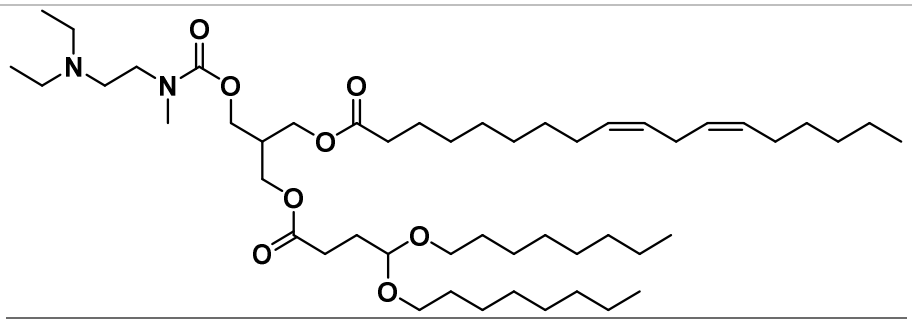


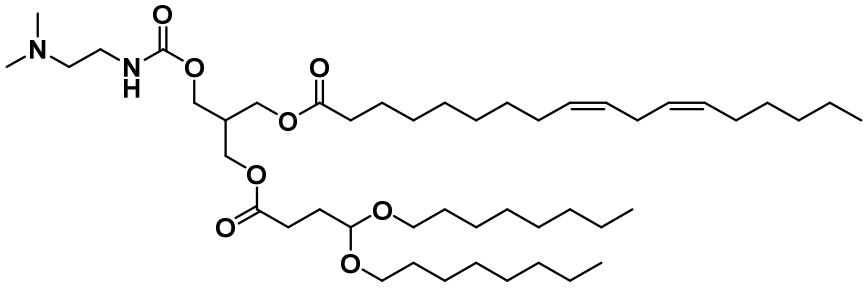


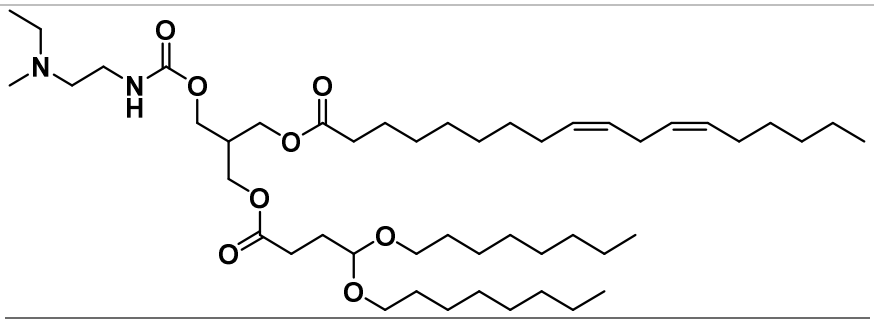


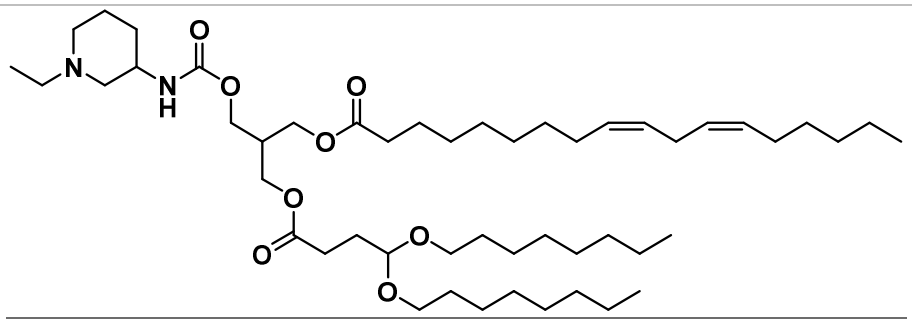


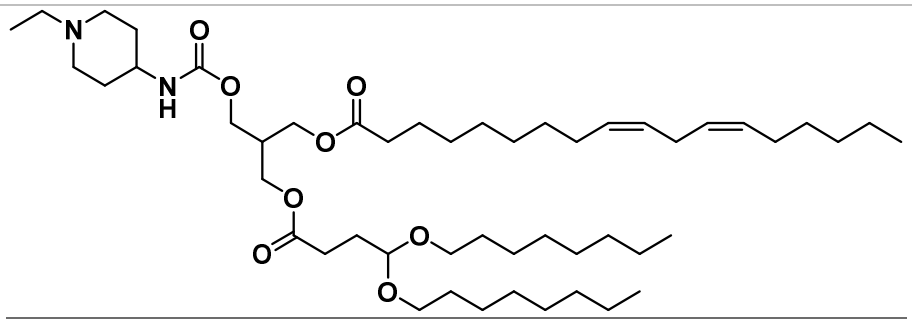


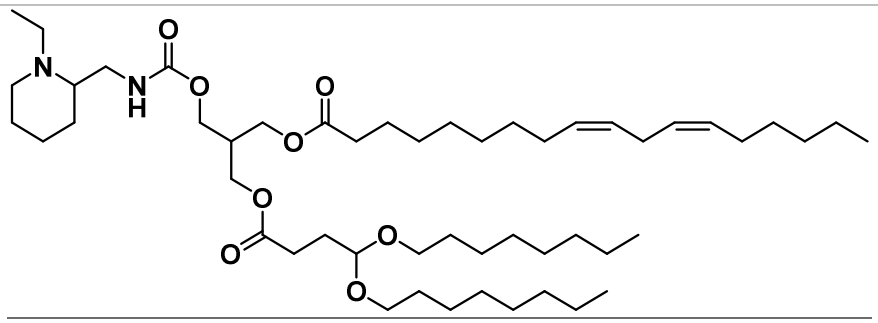


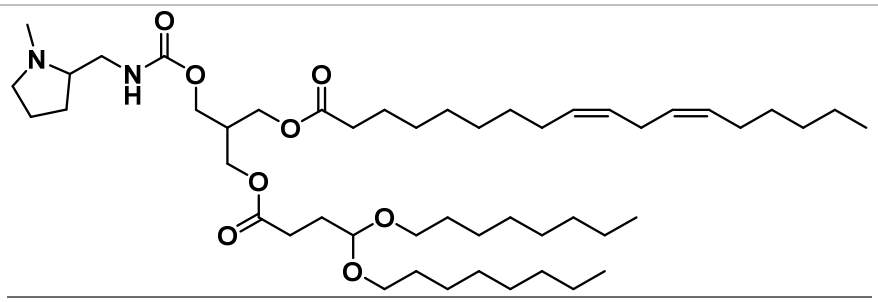


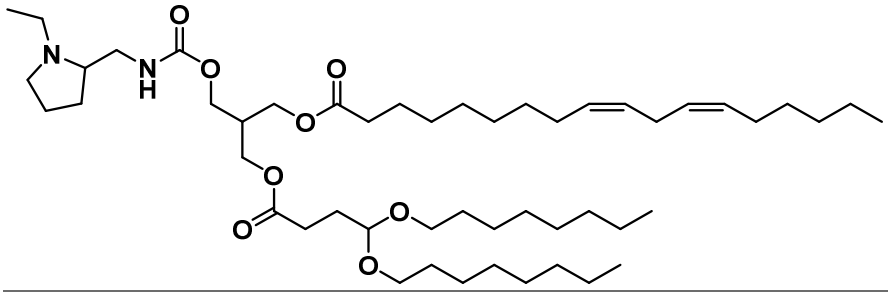


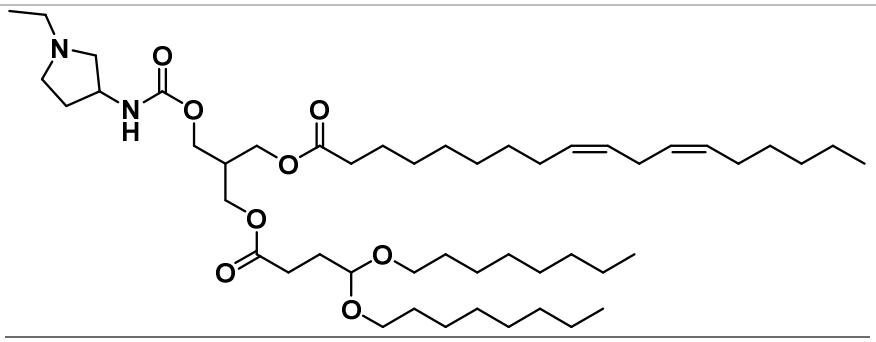


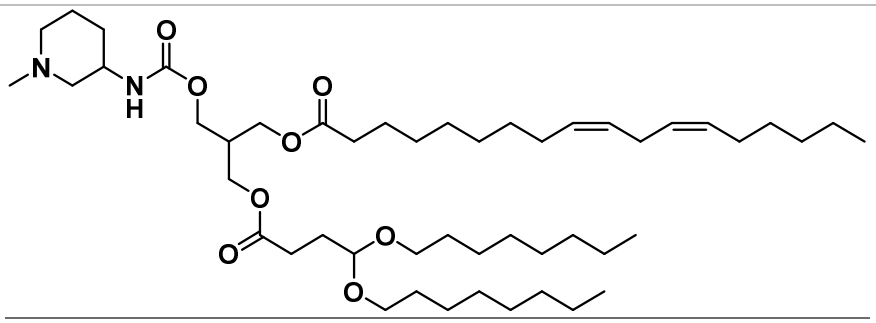


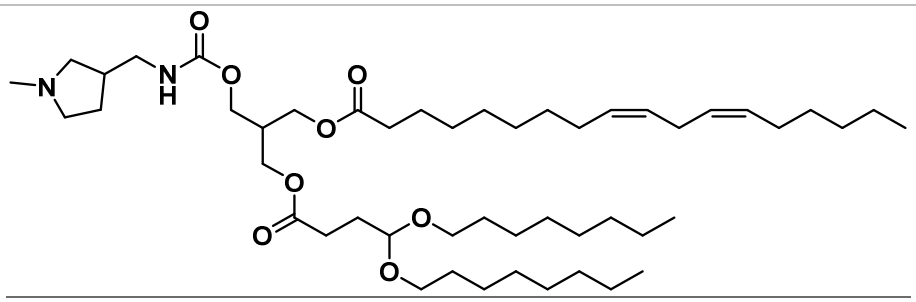


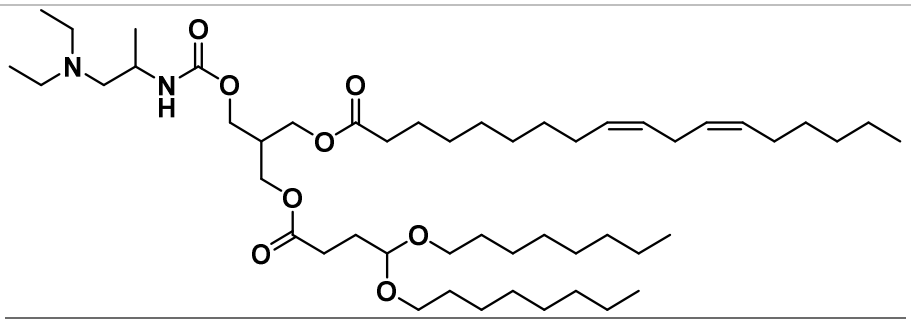


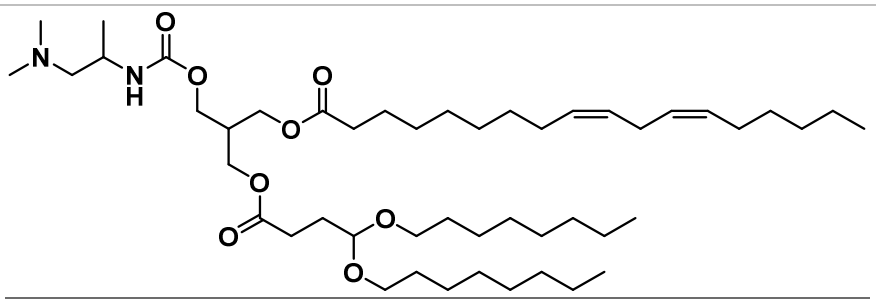


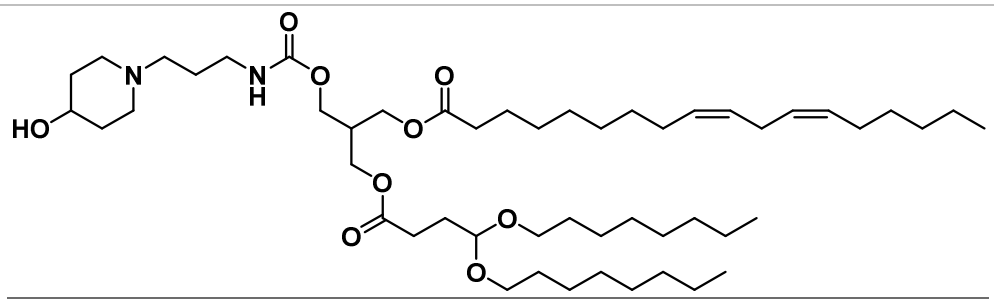


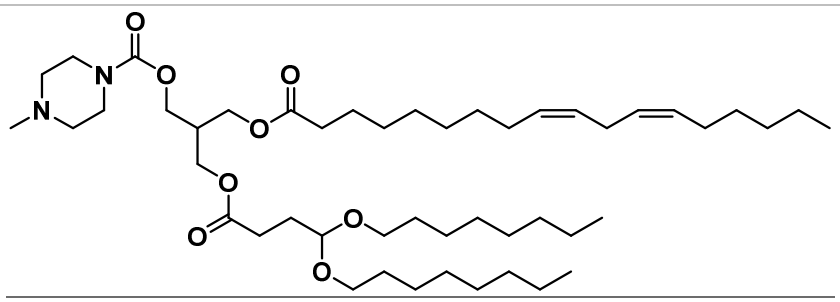


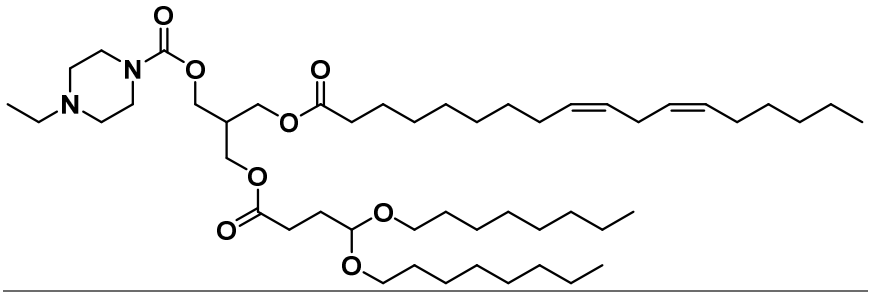


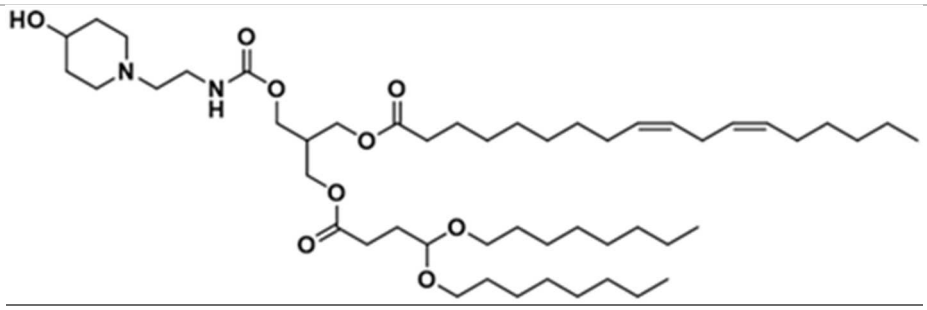


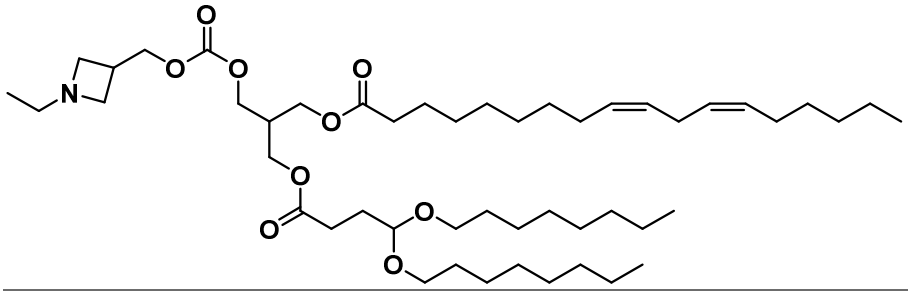


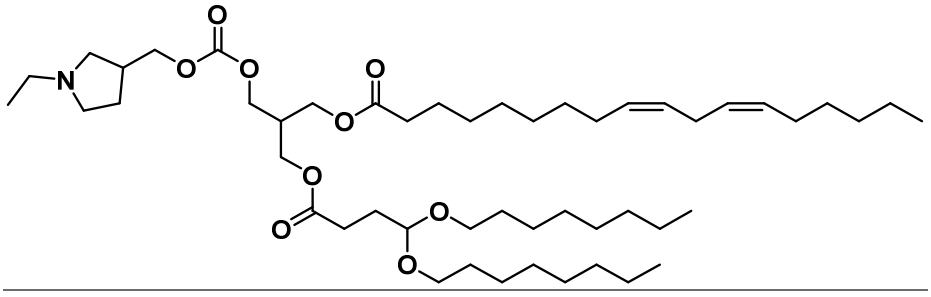


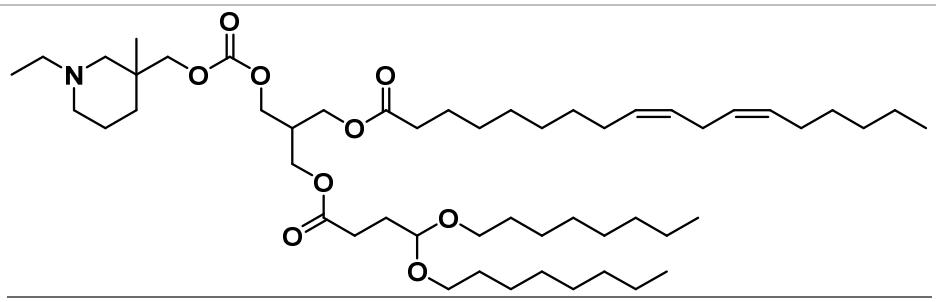


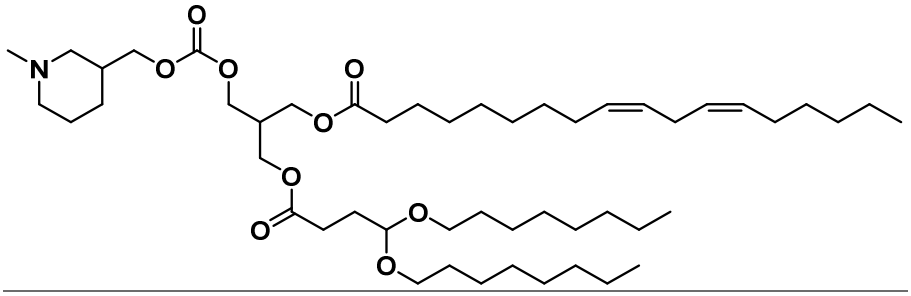


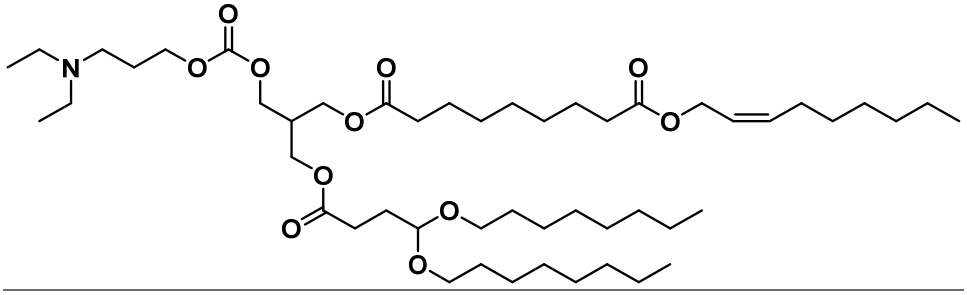


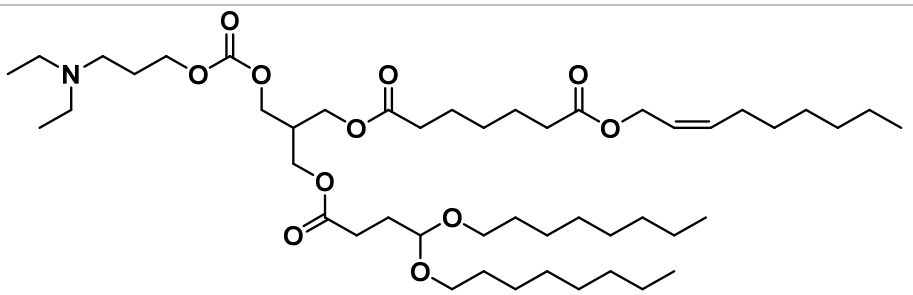


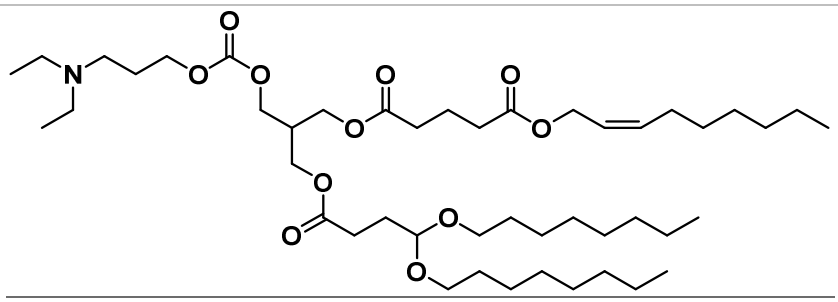


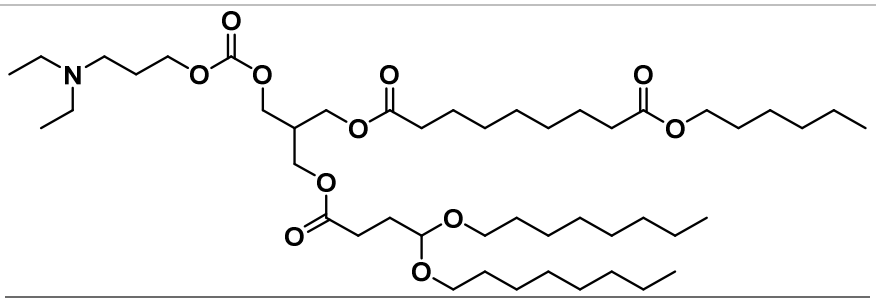


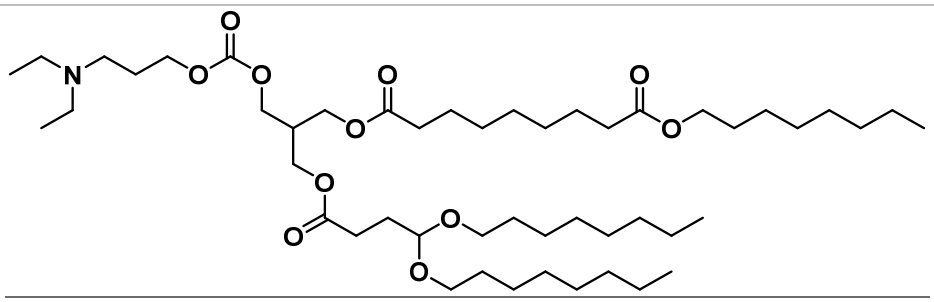


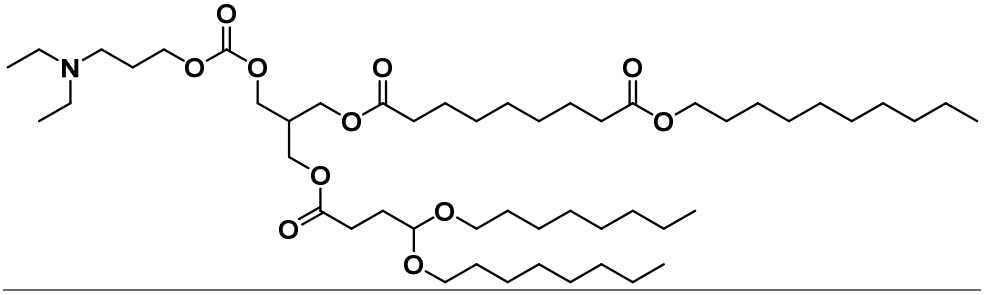


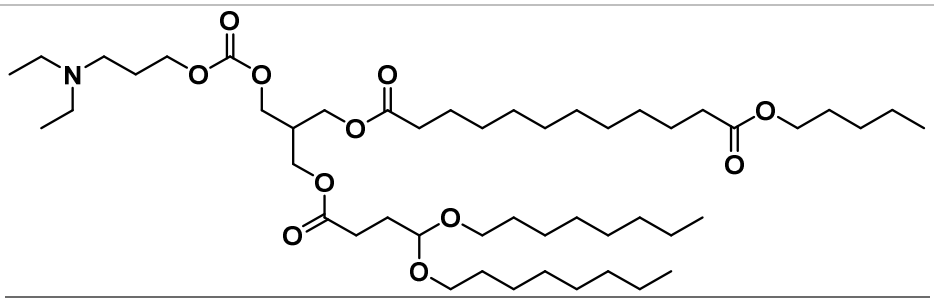


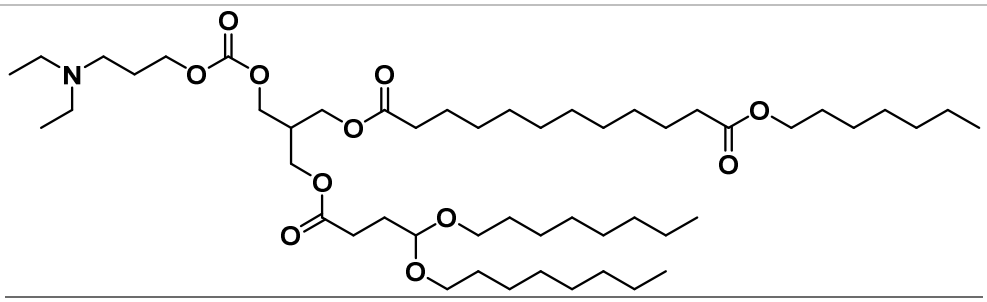


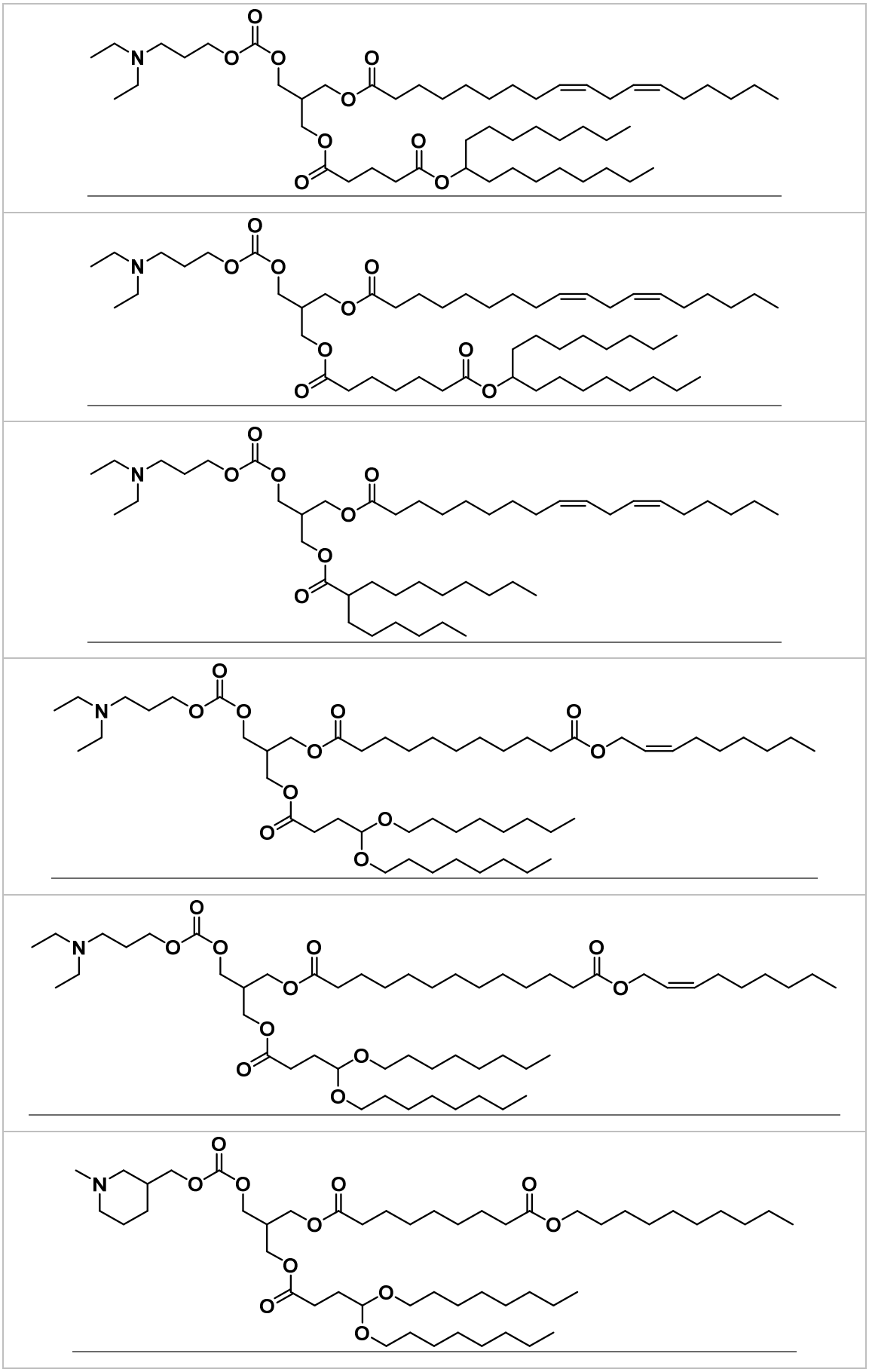


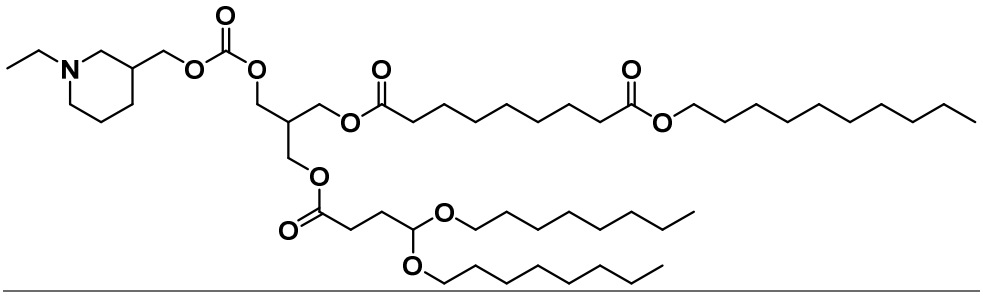


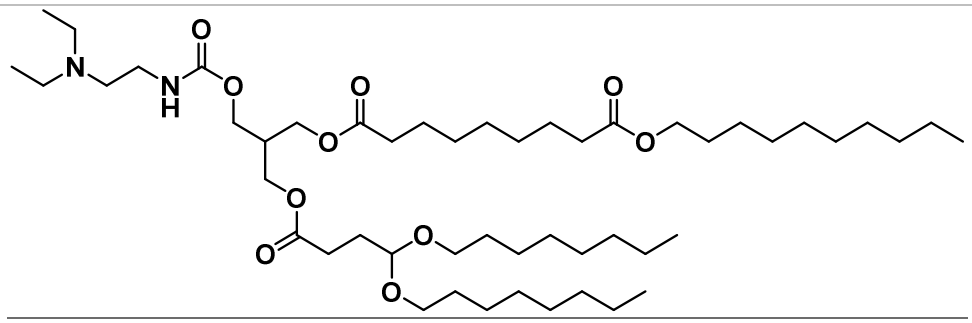


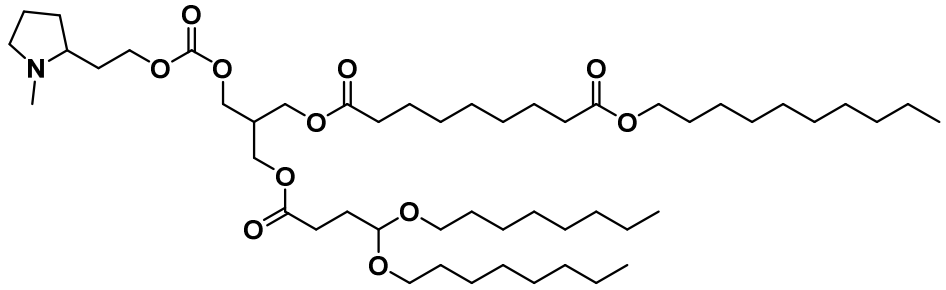


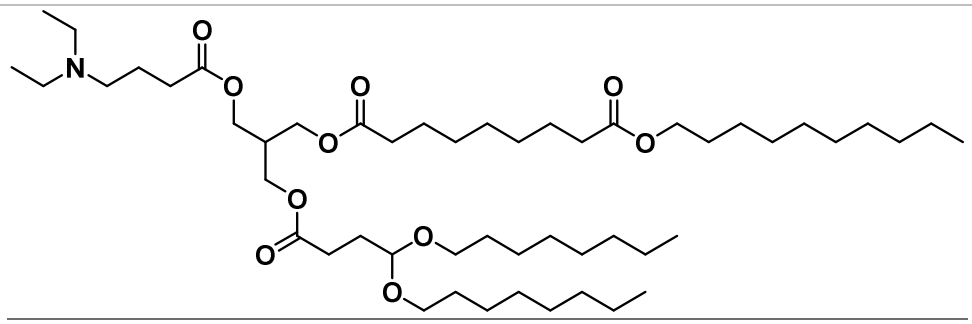


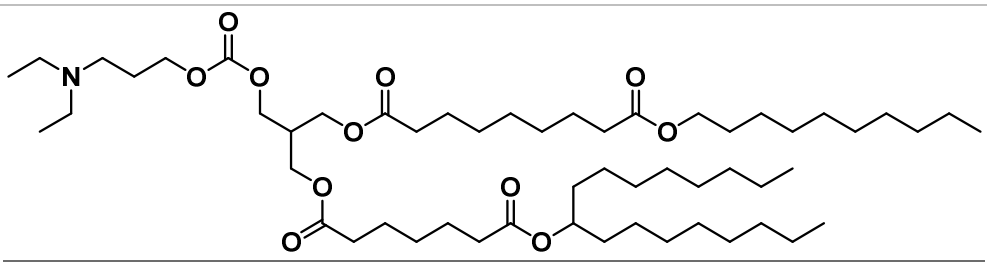


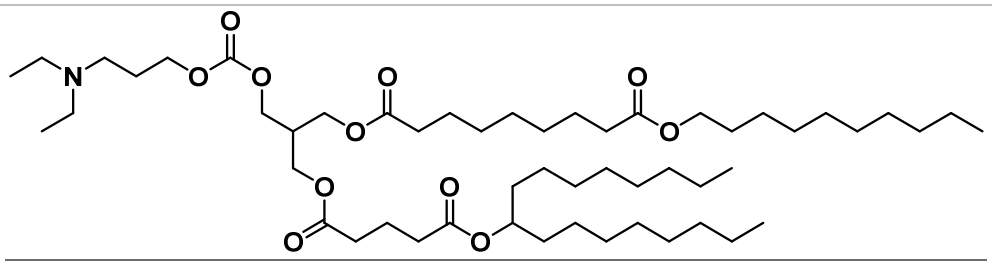


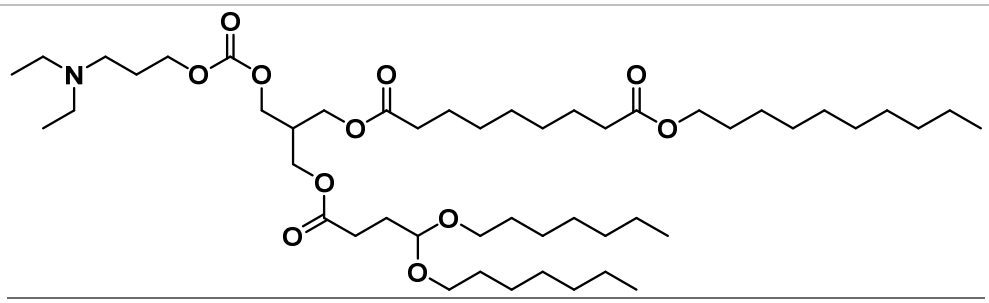


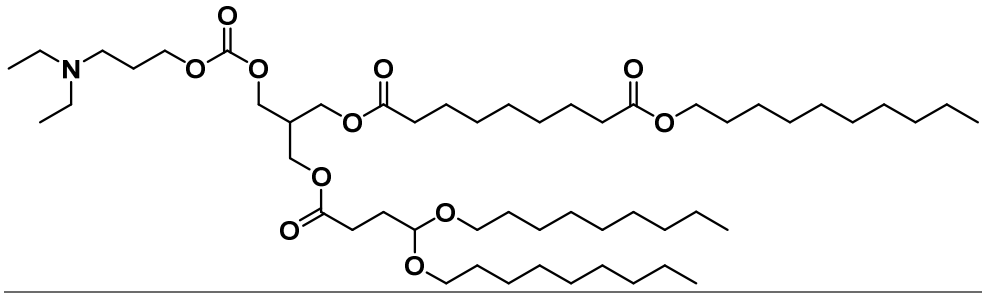


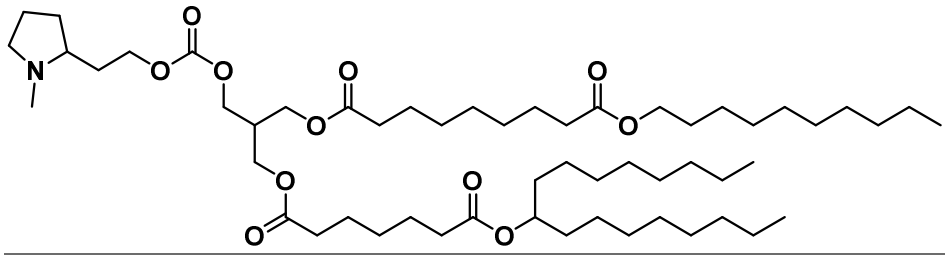


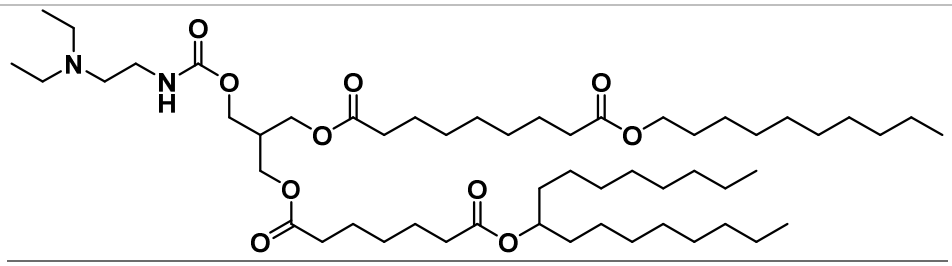


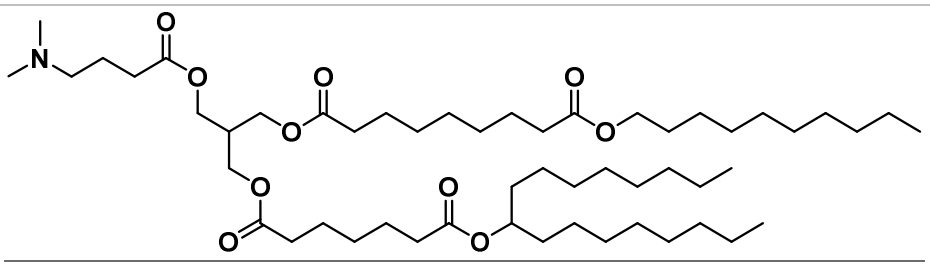


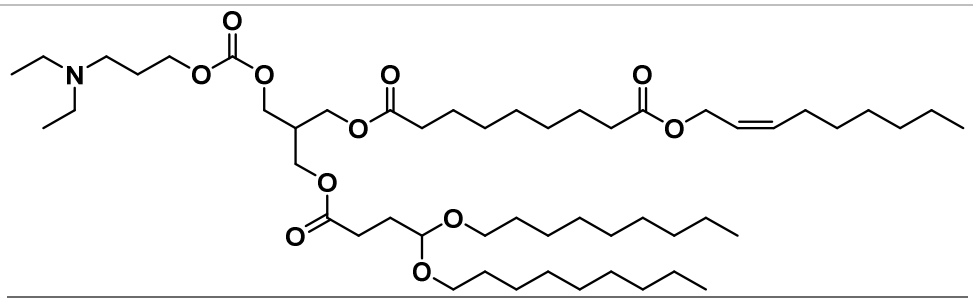


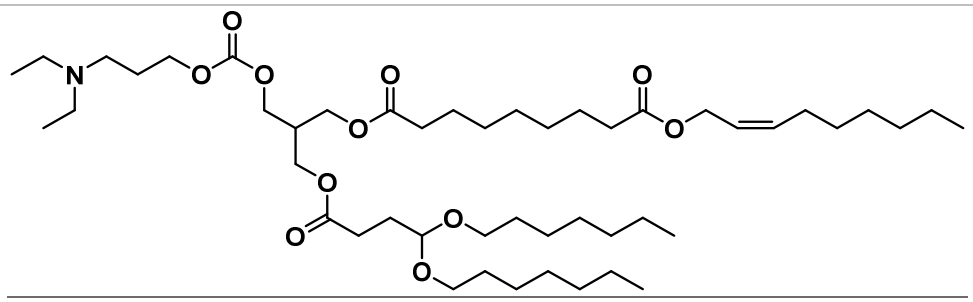


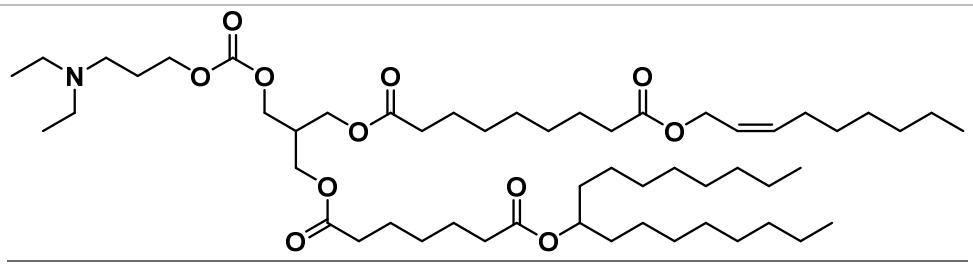


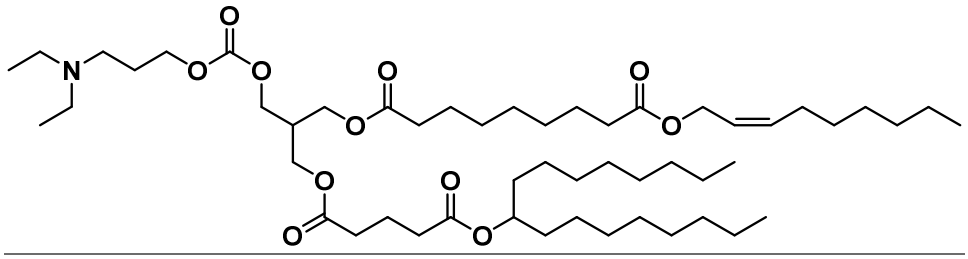


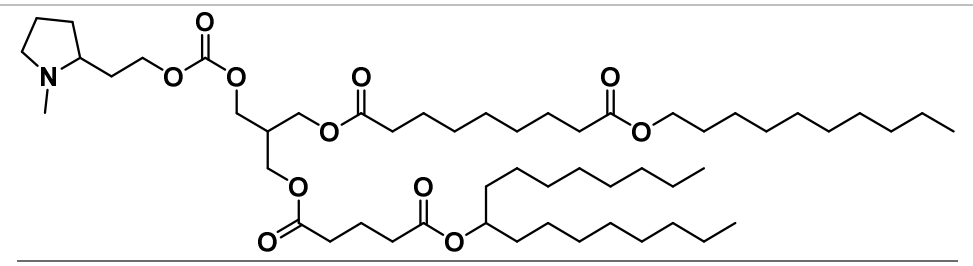


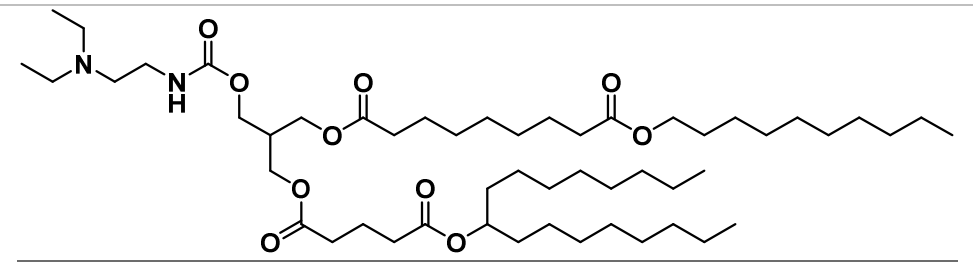


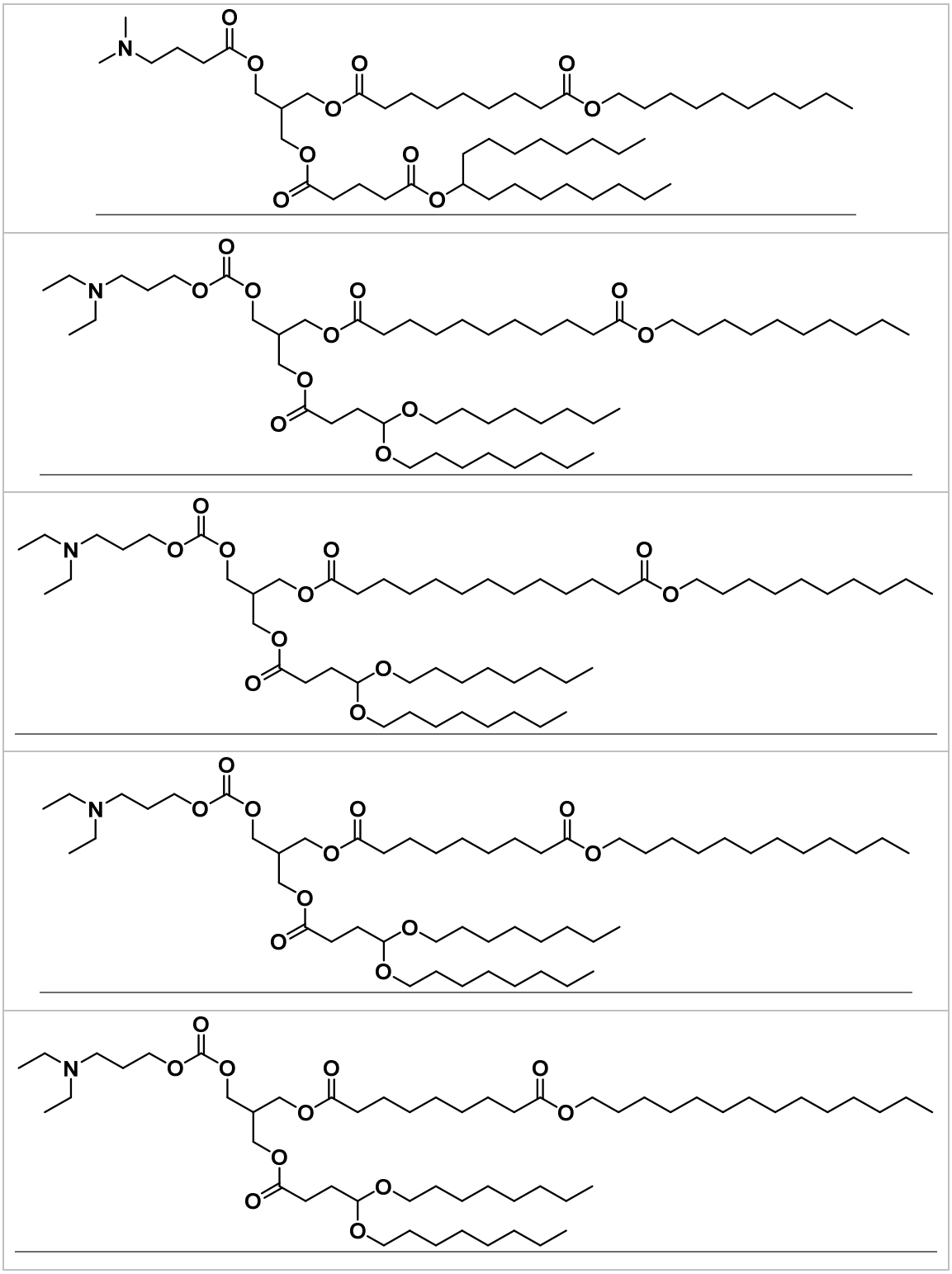


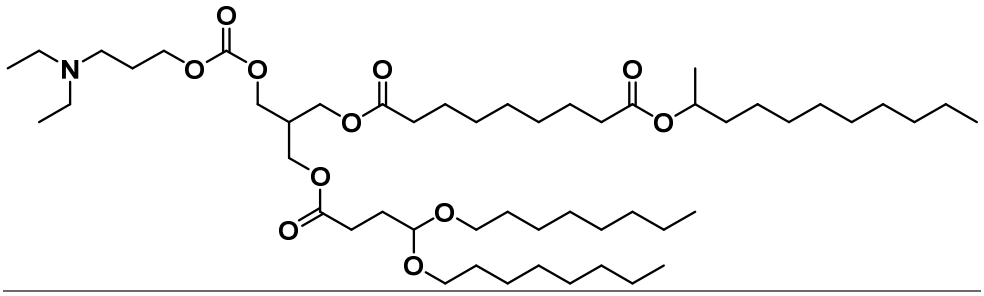


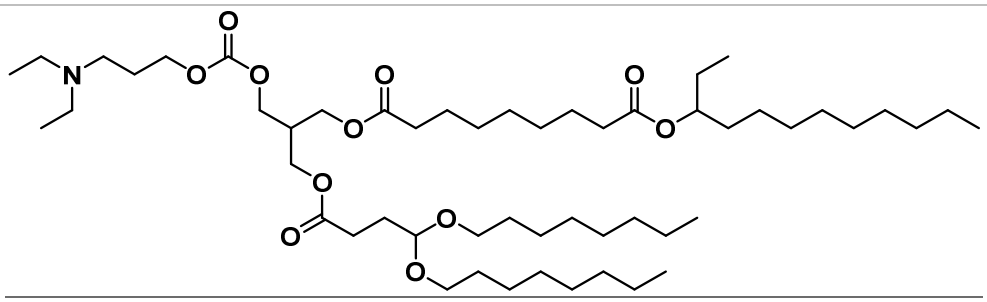


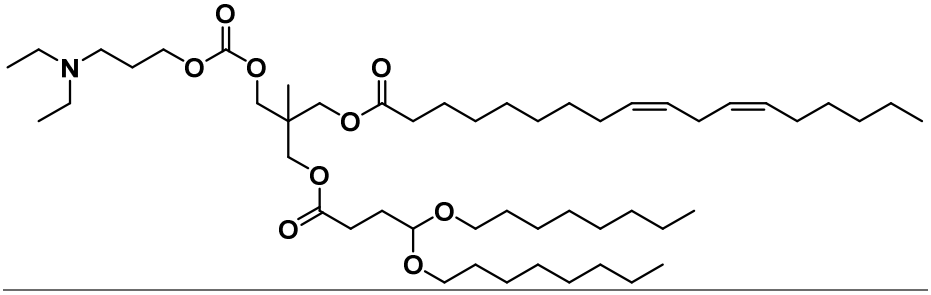


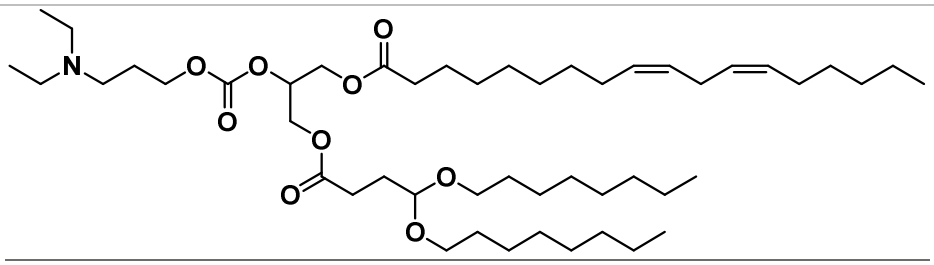


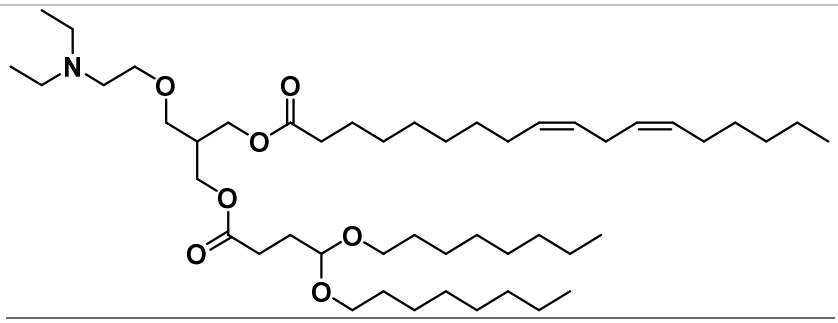


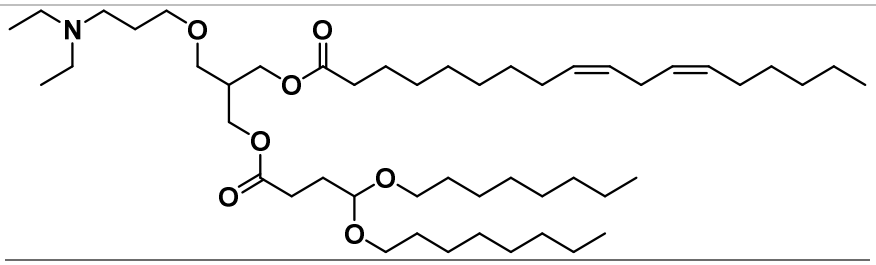


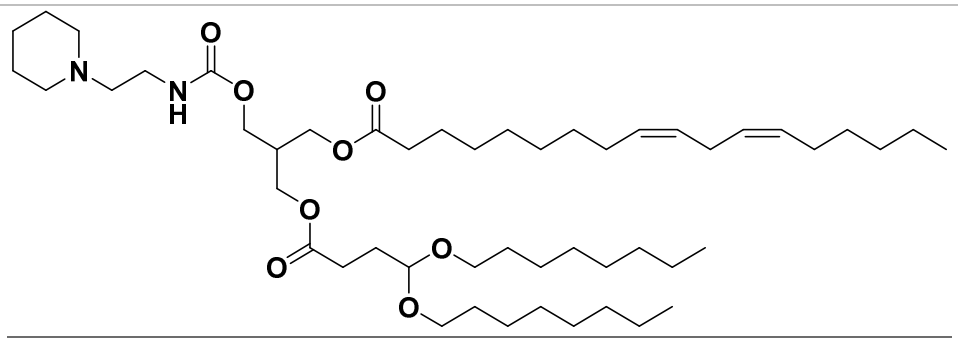


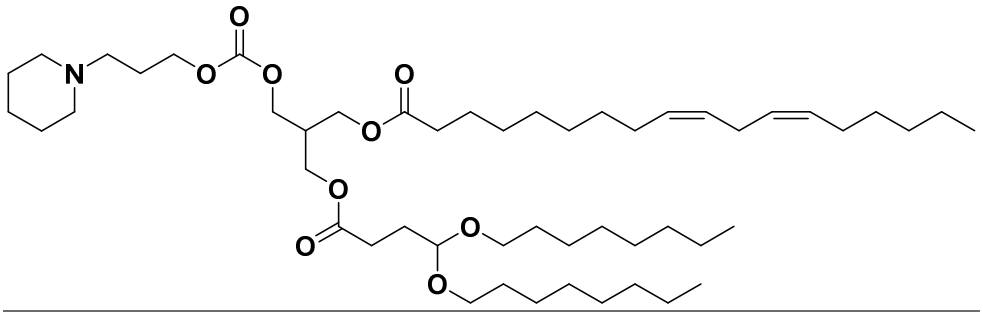


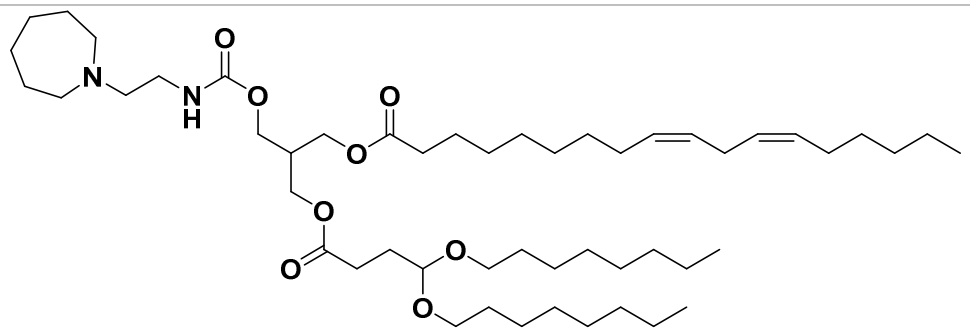


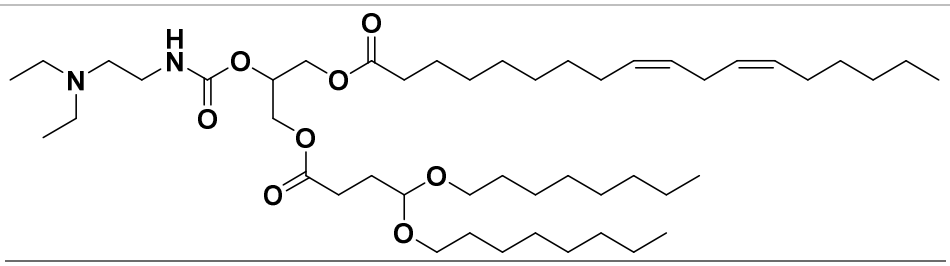


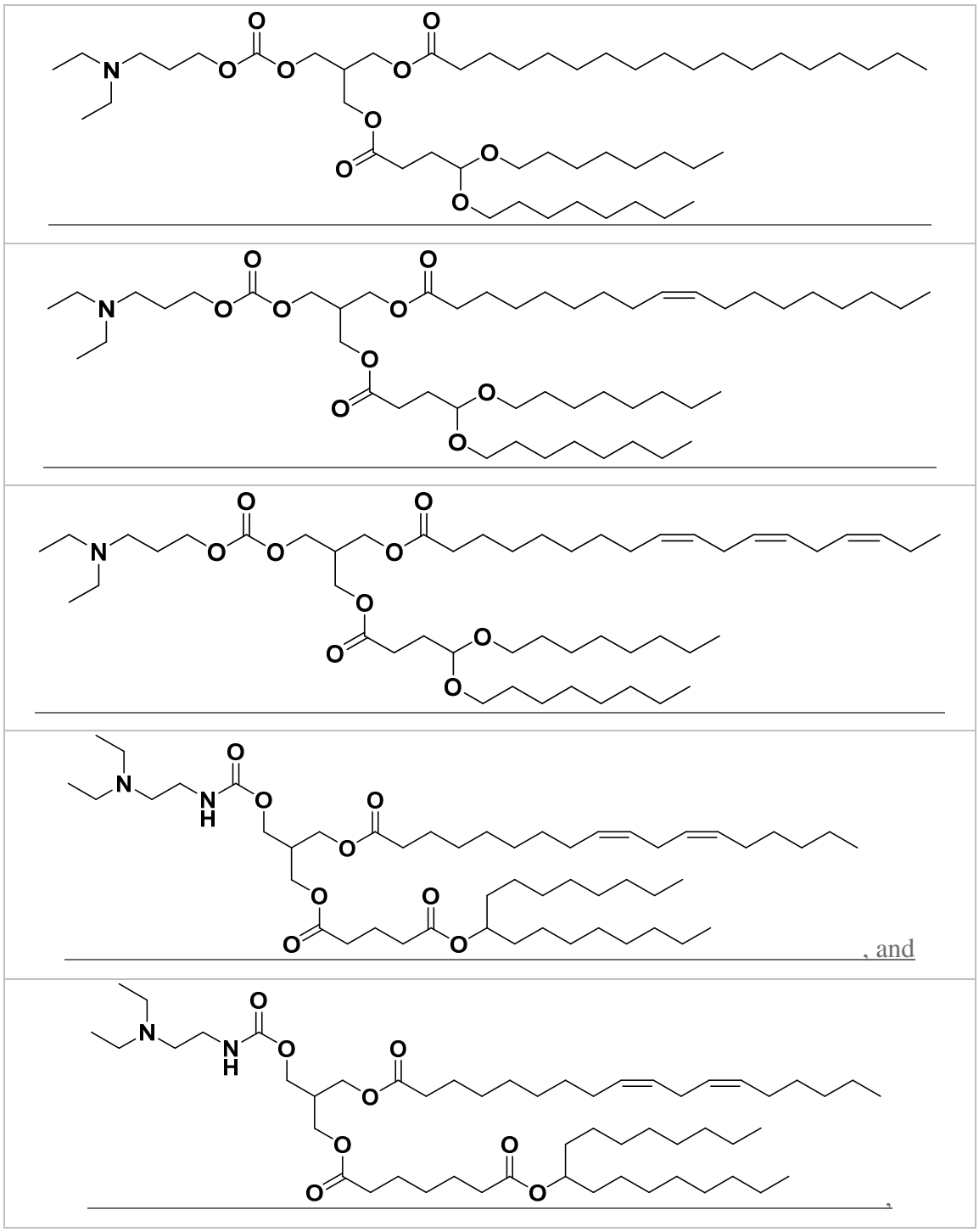












, and

,

or a salt thereof.

19. The compound of any one of claims 1-18, wherein the salt is a pharmaceutically acceptable salt.

20. The compound of any one of the preceding claims, wherein the pKa of the protonated form of the compound is from about 5.1 to about 8.0, from about 5.7 to about 7.6, or from about 6 to about 7.6.

21. A lipid nanoparticle (LNP) composition comprising a lipid component, wherein the lipid component comprises a compound of any one of the preceding claims.

22. The LNP composition of claim 21, wherein the lipid component comprises a helper lipid and a PEG lipid or a helper lipid, a PEG lipid, and a neutral lipid.

23. The LNP composition of claim 21 or 22, wherein the LNP composition comprises an aqueous component comprising a biologically active agent, wherein the biologically active agent comprises a nucleic acid.

24. The LNP composition of claim 23, wherein the nucleic acid comprises RNA.

25. The LNP composition of claim 23 or 24, wherein the LNP composition has an N/P ratio of about 3-10.

26. The LNP composition of claim 25, wherein the N/P ratio is about 6 ± 1.

27. The LNP composition of any one of claims 21 or 22, further comprising an RNA component, wherein the RNA component comprises an mRNA.

28. The LNP composition of claim 27, wherein the RNA component comprises a sequence encoding RNA-guided DNA-binding agent, such as a Cas nuclease mRNA encoding a Class 2 Cas nuclease.

29. The LNP composition of claim 28, wherein the mRNA encodes a Cas9 nuclease.

30. The LNP composition of any one of claims 27-29, wherein the RNA component further comprises a gRNA nucleic acid.

31. The LNP composition of claim 30, wherein the gRNA nucleic acid is a gRNA.

32. The LNP composition of claim 30, wherein the gRNA nucleic acid is or encodes a dual-guide RNA (dgRNA) or a single-guide RNA (sgRNA).

33. The LNP composition of claim 31, wherein the gRNA is a modified gRNA, optionally wherein the modified gRNA comprises a modification at one or more of the first five nucleotides at a 5’ end, and/or optionally wherein the modified gRNA comprises a modification at one or more of the last five nucleotides at a 3’ end.

34. The LNP composition of any one of claims 21-33, further comprising at least one template nucleic acid.

35. Use of a LNP composition of any one of claims 23-34 in the manufacture of a medicament for gene editing in a subject.

36. The LNP composition of any one of claims 23-34 for use in a method of gene editing, the method comprising contacting a cell with the LNP composition.

37. An in vitro method of gene editing, comprising contacting a cell with an LNP composition of any one of claims 23-34.

38. The method of claim 37, wherein the method comprises administering mRNA formulated in a first LNP composition and a second LNP composition comprising one or more of an mRNA, a gRNA, a gRNA nucleic acid, and a template nucleic acid.

39. An in vitro method of cleaving a DNA, comprising contacting a cell with a composition of any one of claims 23-34.

40. The method of claim 39, wherein the contacting step results in a single stranded DNA nick.

41. The method of claim 39 or 40, further comprising introducing at least one template nucleic acid into the cell.

42. The method of any one of claims 37-41, wherein the cell is a eukaryotic cell.