

TRICINE AND CITRIC ACID-BASED CATIONIC LIPIDS

RELATED APPLICATIONS

This application claims priority to European application no. EP23305930.2 filed on 12th June 2023, European application no. EP23306900.4 filed on 2nd November 2023, and European application
5 no. EP23306902.0 filed on 2nd November 2023, the entire disclosures of which are hereby incorporated by reference.

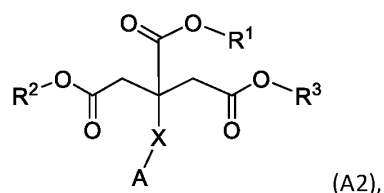
BACKGROUND

[001] Delivery of nucleic acids has been explored extensively as a potential therapeutic option for certain disease states. In particular, messenger RNA (mRNA) therapy has become an
10 increasingly important option for treatment of various diseases, including for those associated with deficiency of one or more proteins.

SUMMARY OF THE INVENTION

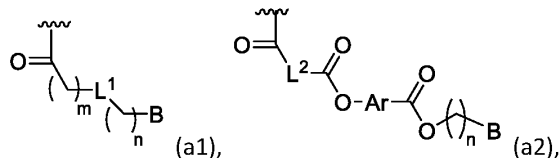
[002] The present invention provides, among other things, a novel class of tricine and citric
15 acid-based cationic lipid compounds for improved *in vivo* delivery of therapeutic agents, such as nucleic acids. It is contemplated that the compounds provided herein can be capable of highly effective *in vivo* delivery while maintaining a favorable toxicity profile. It is contemplated that the compounds provided herein can be capable of highly effective intranasal delivery. Indeed, Examples 43 and 45 shows that lipid nanoparticles comprising compounds of the present
20 invention (e.g. compound 64 or a compound in Table 6, or a pharmaceutically acceptable salt thereof) are particularly effective at intranasal delivery.

[003] In one aspect, the invention features a cationic lipid having a structure according to Formula (A2):

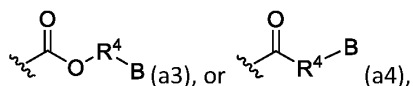


25 or a pharmaceutically acceptable salt thereof, wherein
X is independently O or NH;

each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, C_4 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;



A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L^1 is a carbonyl, ester, or amide;

L^2 is C_2 - C_{10} alkylene or a C_2 - C_{10} alkenylene;

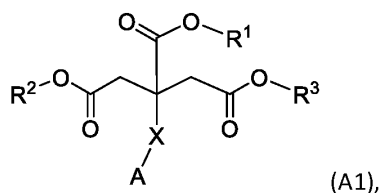
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 ;

R^4 is C_1 - C_{10} alkylene;

and wherein **A** is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently a C_4 -

C_{30} heteroalkyl comprising a disulfide bond or having a structure that is $\text{---}(\text{---})_o\text{---}L^3\text{---}R^5$,
 wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; **o** is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; **o** is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

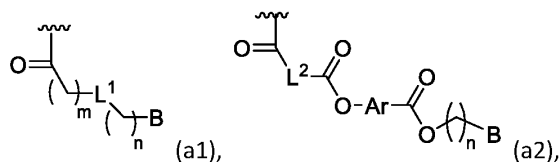
[004] In one aspect, the invention features a cationic lipid having a structure according to Formula (A1):



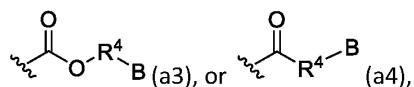
or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, C_4 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;



A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

5 **m** is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

Ar is phenylene optionally comprising 1-4 substituents independently selected from

10 halogen, OCH₃, and CH₃;

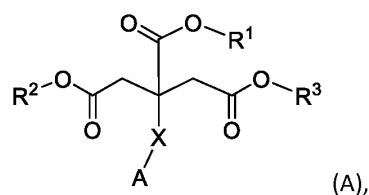
R⁴ is C₁-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R¹**, **R²**, and **R³** is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is ,

15 wherein **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl; or **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₅ alkyl.

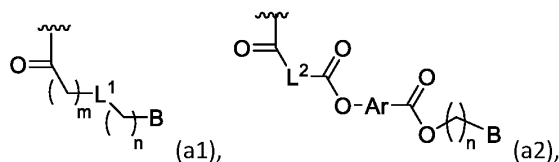
[005] In one aspect, the invention features a cationic lipid having a structure according to Formula (A):



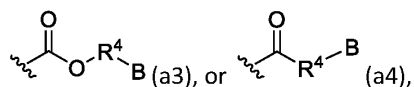
or a pharmaceutically acceptable salt thereof, wherein

20 **X** is independently O or NH;

each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;



A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

5 **m** is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

Ar is phenylene optionally comprising 1-4 substituents independently selected from

10 halogen, OCH₃, and CH₃;

R⁴ is C₂-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R¹**, **R²**, and **R³** is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is ,
wherein **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl; or **L³** is

15 OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₅ alkyl.

[006] In embodiments, **X** is O and/or **B** is independently an ionizable nitrogen-containing group.

[007] In embodiments, each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, or C₄-C₃₀ alkynyl.

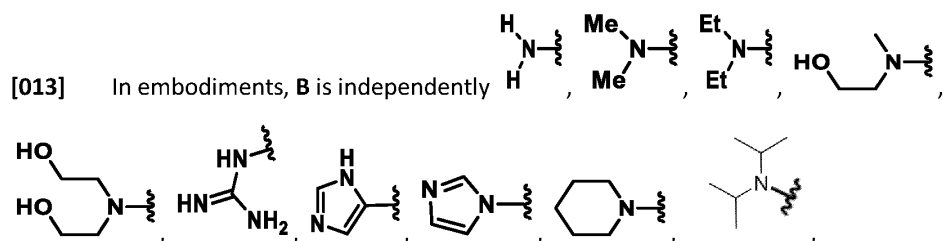
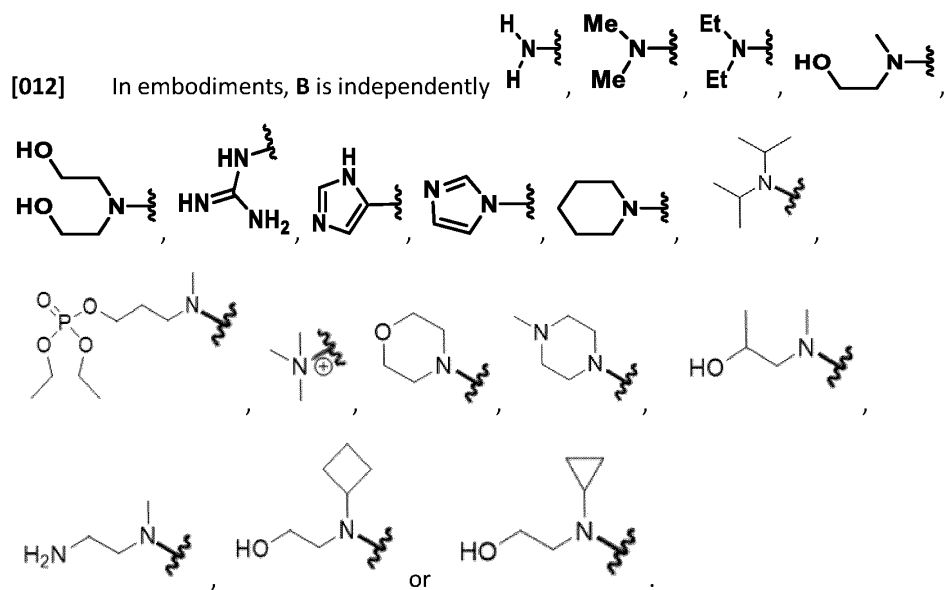
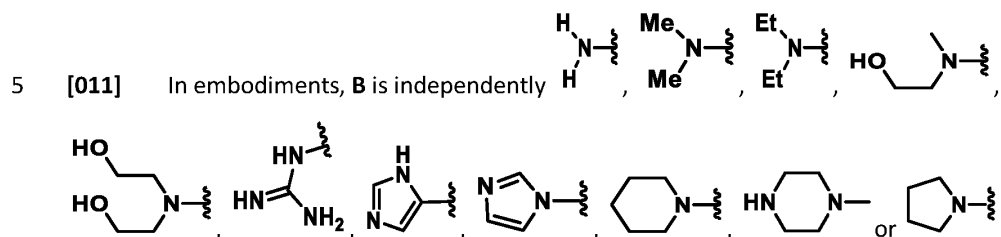
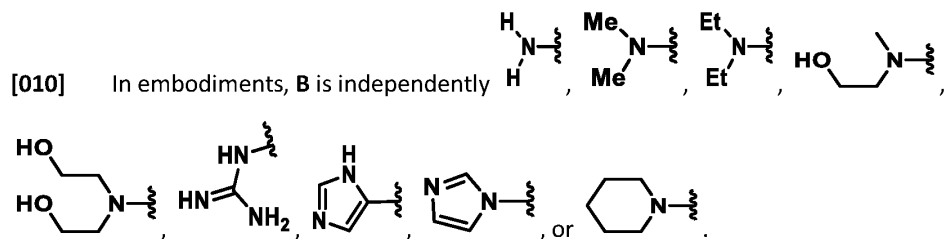
20 [008] In embodiments of Formula (A2), each of **R¹**, **R²**, and **R³** is independently a C₄-C₃₀

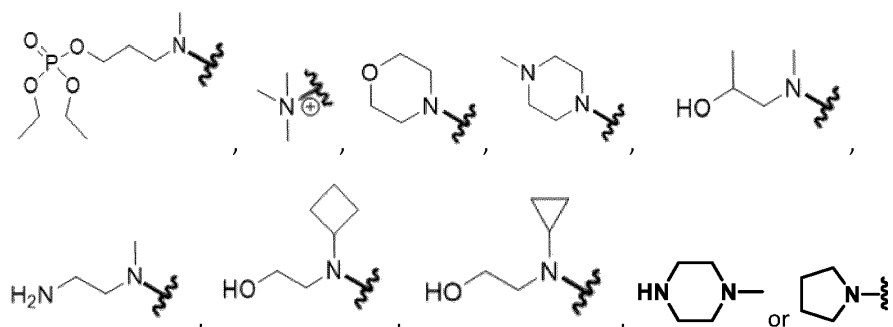
heteroalkyl comprising a disulfide bond or having a structure that is , wherein **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl; or **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₆ alkyl.

[009] In embodiments, each of **R¹**, **R²**, and **R³** is independently a C₄-C₃₀ heteroalkyl comprising

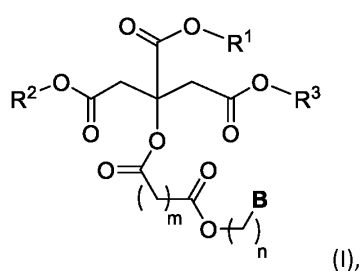
25 a disulfide bond or having a structure that is , wherein **L³** is OC(O), CO₂, or (O)CO; **o** is

an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.





[014] In embodiments, a compound has a structure according to Formula (I):



5 or a pharmaceutically acceptable salt thereof, wherein

each of **R¹**, **R²**, and **R³** is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

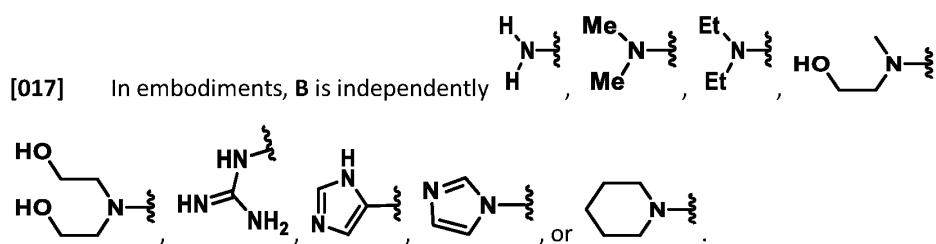
m is an integer of 2-10;

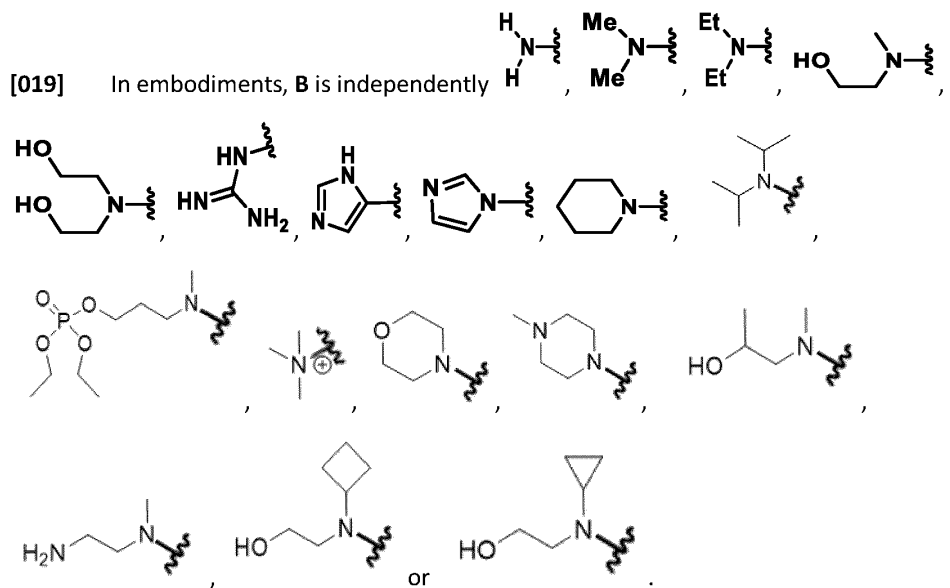
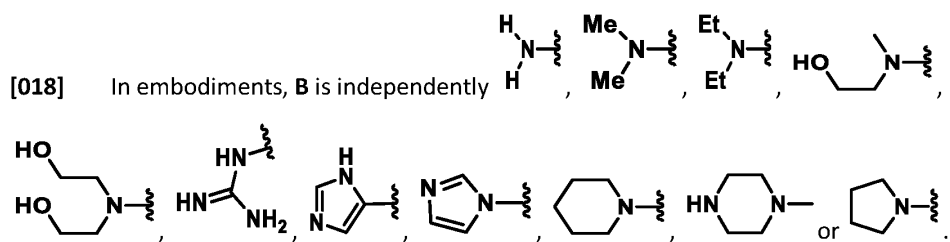
n is an integer of 2-10; and

10 **B** is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group.

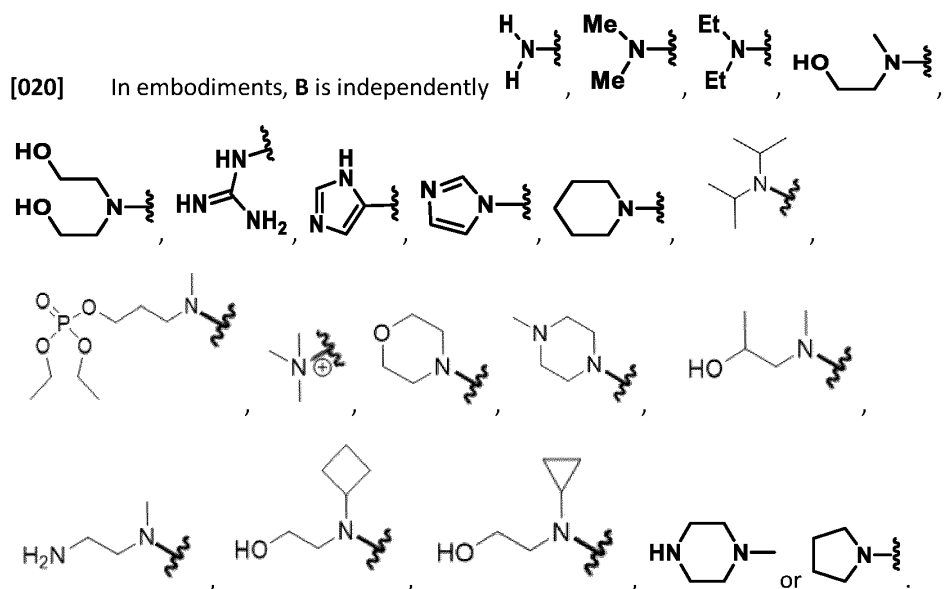
[015] In embodiments, **B** is independently an ionizable nitrogen containing group.

[016] In embodiments, m is 2 and/or n is 2 or 3.

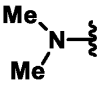
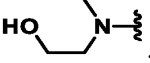




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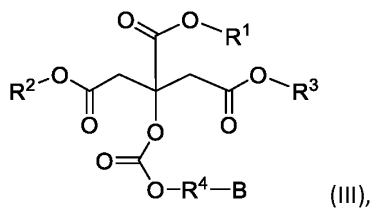


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[021] In embodiments, **B** is  or .

[022] In embodiments, a compound is selected from the group consisting of: Compounds (1), (2), (3), (4), (6), (9), (17), (24), (26), (27), (33), and (35).

[023] In embodiments, a compound has a structure according to Formula (III):



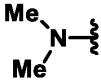
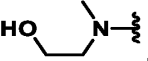
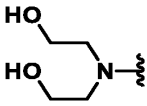
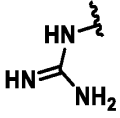
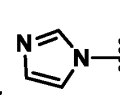
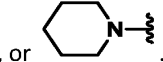
or a pharmaceutically acceptable salt thereof, wherein

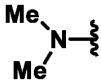
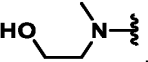
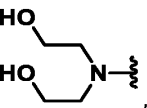
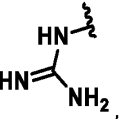
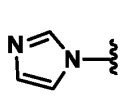
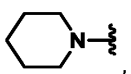
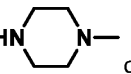
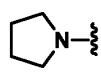
each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

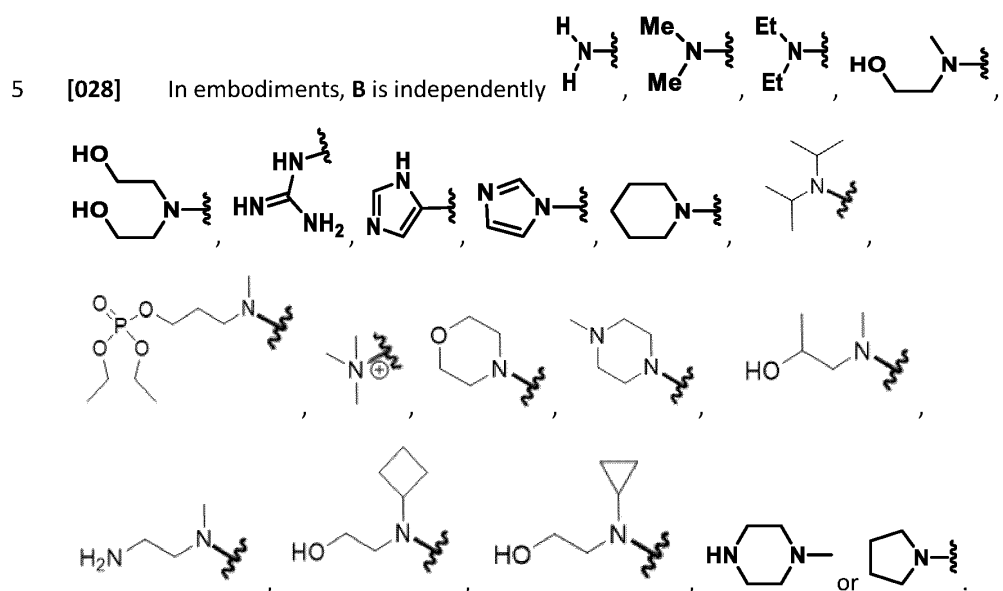
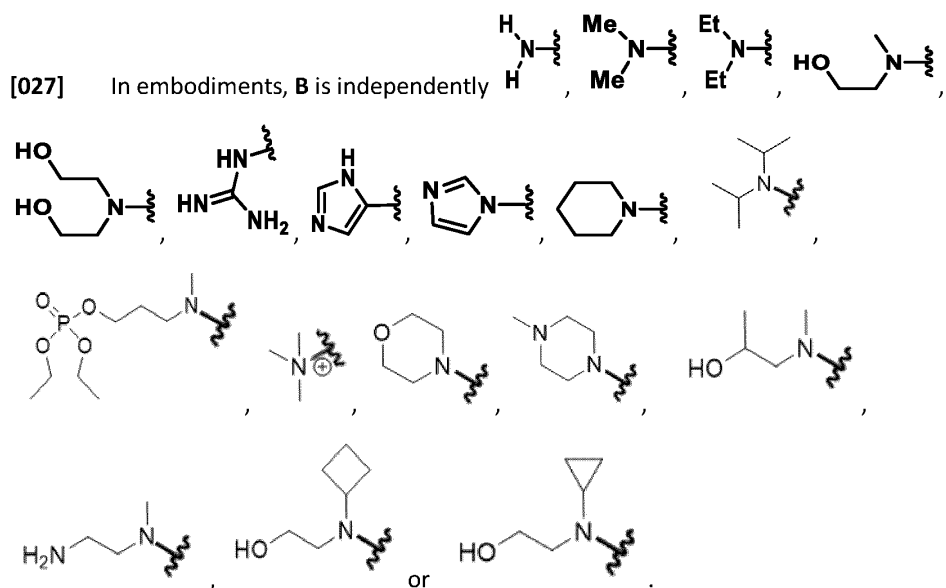
R⁴ is C₂-C₁₀ alkylene; and

B is independently an ionizable nitrogen-containing group.

[024] In embodiments, **R**⁴ is -CH₂CH₂-.

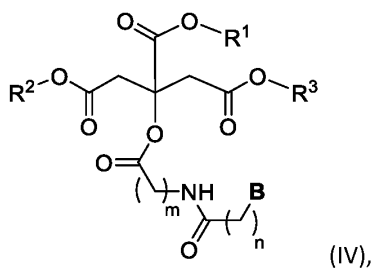
[025] In embodiments, **B** is independently , , , , , , , , or .

[026] In embodiments, **B** is independently , , , , , , , , , , or .



10 [030] In embodiments, a compound is Compound (3).

[031] In embodiments, a compound has a structure according to Formula (IV):



or a pharmaceutically acceptable salt thereof, wherein

each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

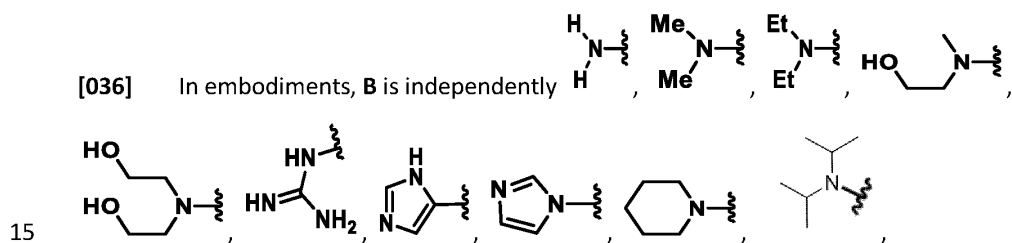
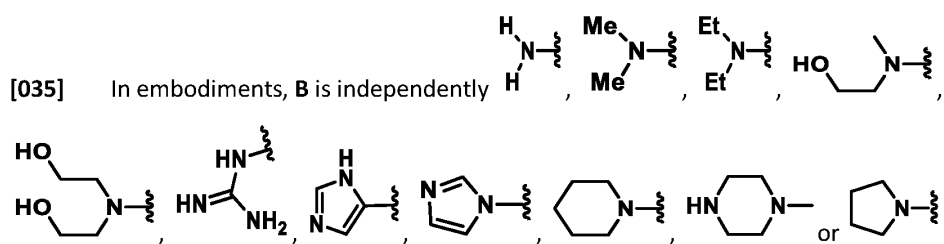
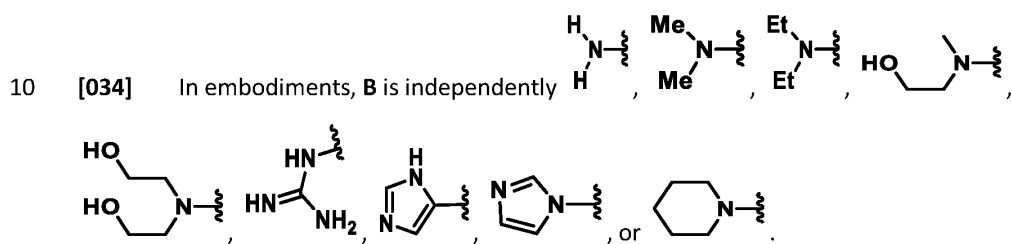
5 **m** is an integer of 2-10;

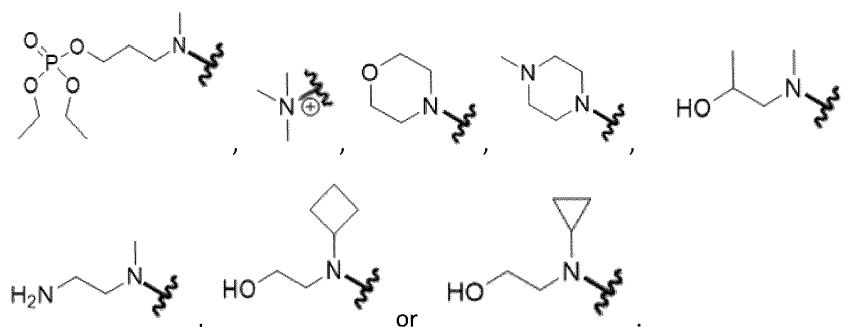
n is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group.

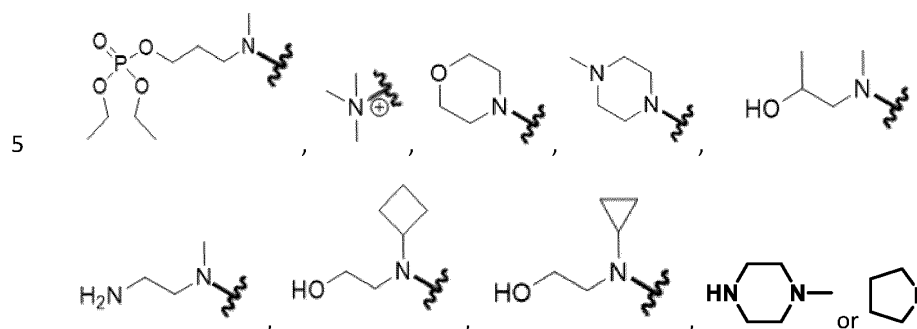
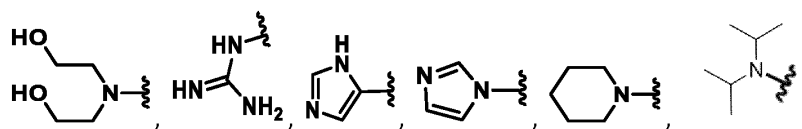
[032] In embodiments, **R**¹, **R**², and **R**³ is C₄-C₃₀ heteroalkyl.

[033] In embodiments, **m** is 2 and/or **n** is 2 or 3.





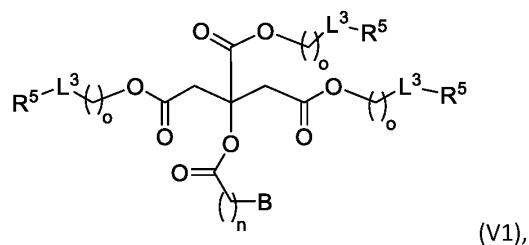
[037] In embodiments, **B** is independently $\text{H-N}(\text{R})_2$, $\text{Me-N}(\text{R})_2$, $\text{Et-N}(\text{R})_2$, $\text{HO-CH}_2\text{CH}_2\text{N}(\text{R})_2$,



[038] In embodiments, **B** is $\text{Me-N}(\text{R})_2$.

[039] In embodiments, a compound is Compound (8) or (39).

[040] In embodiments, a compound has a structure according to Formula (V1):



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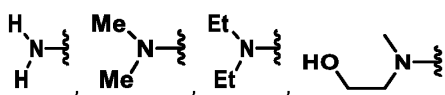
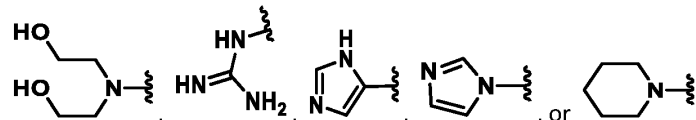
or a pharmaceutically acceptable salt thereof, wherein

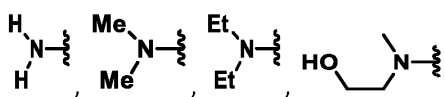
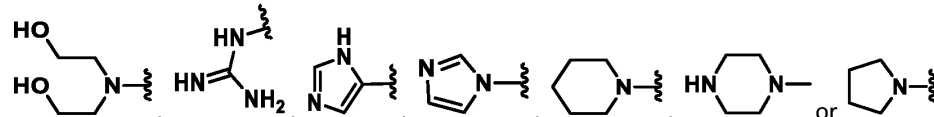
n is an integer of 2, 3, 4, 5, 6, or 7; and

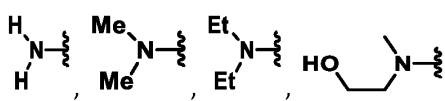
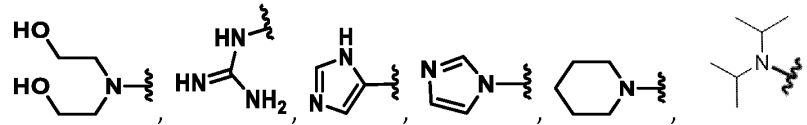
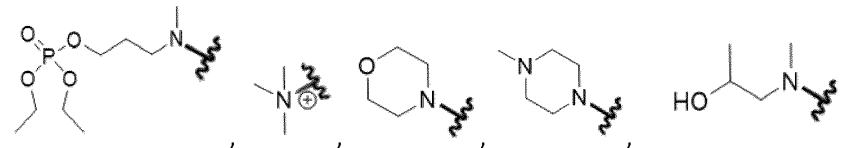
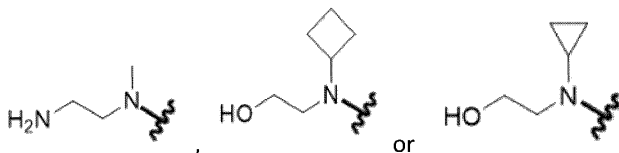
B is independently an ionizable nitrogen-containing group.

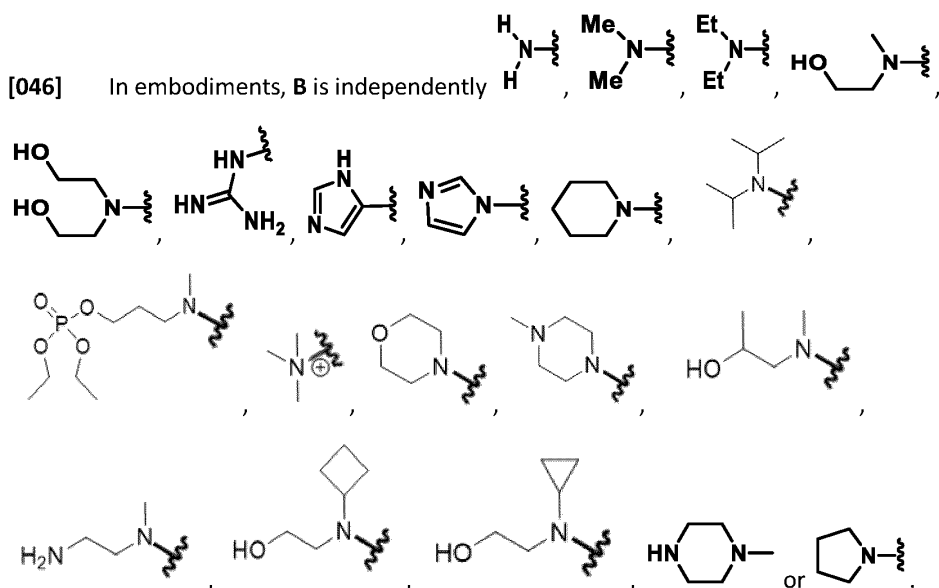
[041] In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; α is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl.

[042] In embodiments of Formula (A1), Formula (A), and Formula (V1), L^3 is $OC(O)$, CO_2 , or $(O)CO$; α is an integer of 6-12; and R^5 is C_1 - C_5 alkyl. In embodiments of Formula (A2) and Formula (V1), L^3 is $OC(O)$, CO_2 , or $(O)CO$; α is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

[043] In embodiments, **B** is independently , .

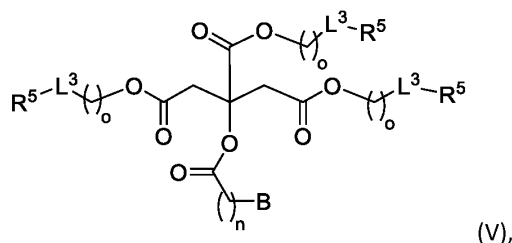
[044] In embodiments, **B** is independently , .

10 [045] In embodiments, **B** is independently , , , .



[048] In embodiments, a compound is selected from the group consisting of: Compounds (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), (40), (112), (113), (114), (115), (116), (117), (126), (127), (128), and (129).

[049] In embodiments, a compound has a structure according to Formula (V):



10

or a pharmaceutically acceptable salt thereof, wherein

n is an integer of 2, 3, or 4; and

B is independently an ionizable nitrogen-containing group.

[050] In embodiments, **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl.

15 [051] In embodiments of Formula (A1), Formula (A), and Formula (V), **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₅ alkyl. In embodiments of Formula (A2) and Formula (V), **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₆ alkyl

[052] In embodiments, B is independently

[053] In embodiments, **B** is independently

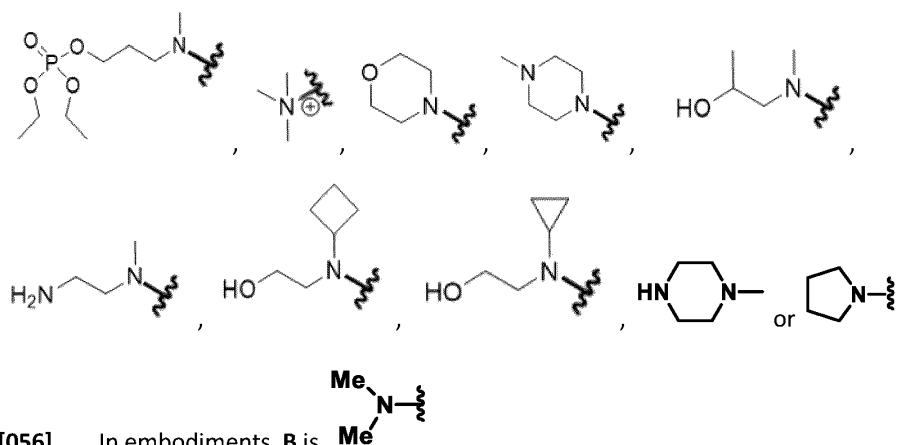


[054] In embodiments, B is independently

[054] In embodiments, B is independently

[055] In embodiments, B is independently

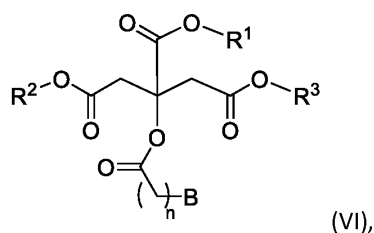




5 (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), (40), (114), and (116).

[058] In embodiments, a compound is selected from the group consisting of: Compounds (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), and (40).

[059] In embodiments, a compound has a structure according to Formula (VI):

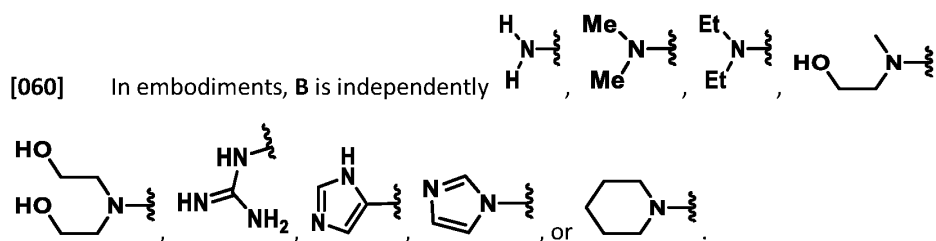


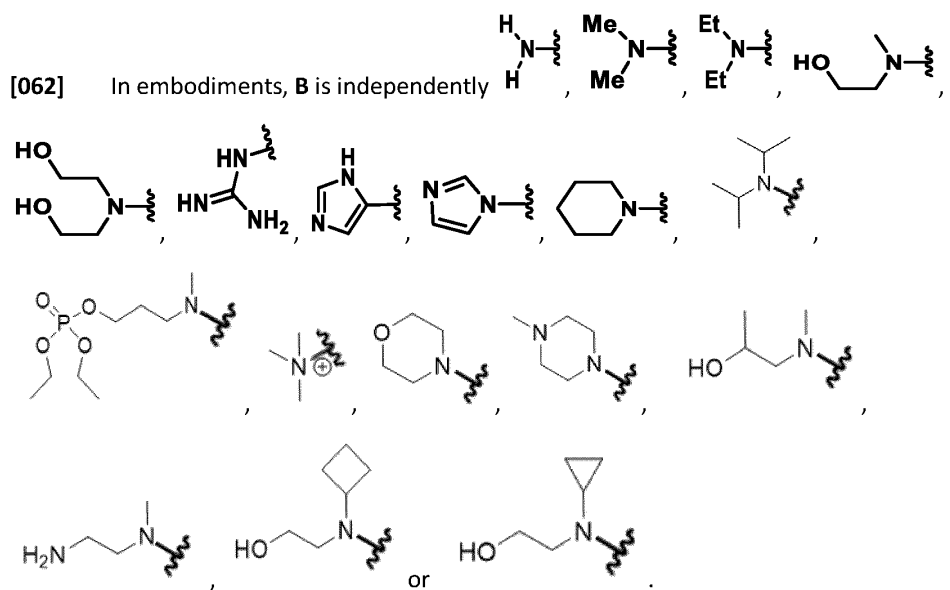
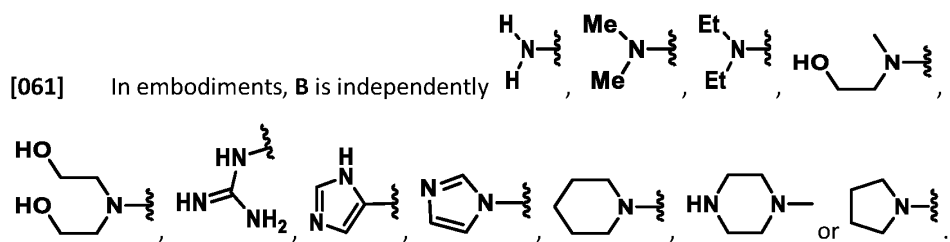
10 or a pharmaceutically acceptable salt thereof, wherein

each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ heteroalkyl comprising a disulfide group;

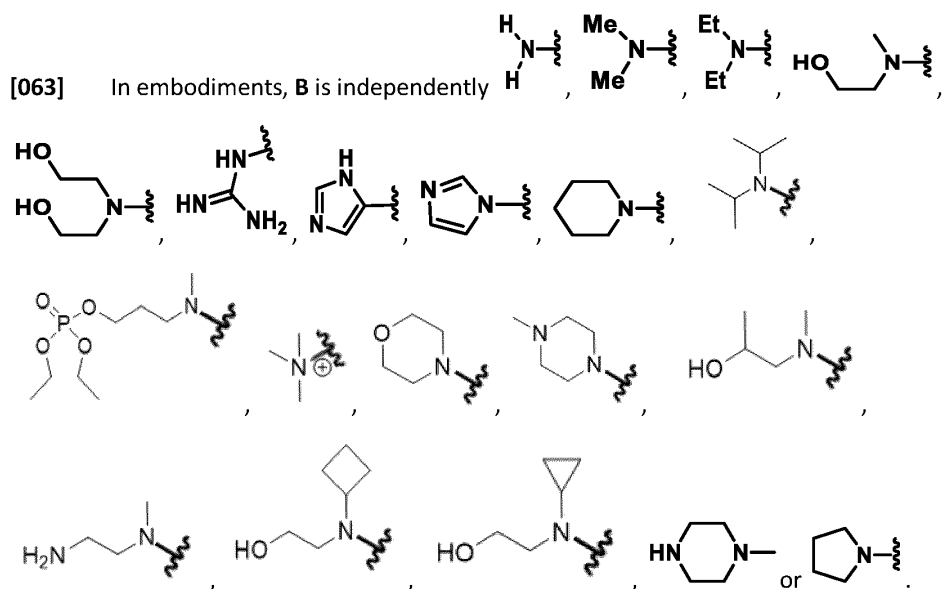
n is an integer of 2, 3, or 4; and

B is independently an ionizable nitrogen-containing group.

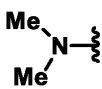




5



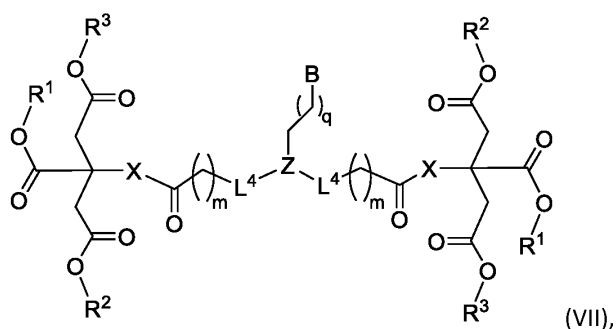
10



[064] In embodiments, **B** is .

[065] In embodiments, a compound is Compound (21).

[066] In embodiments, a compound has a formula according to Formula (VII),



5 or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

each **m** is an integer of 2-10;

10 each **L⁴** is a carbonyl, ester, or amide;

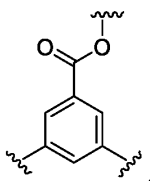
Z is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, wherein **q₁** and **q₂** are independently integers of 2-10; or **Z** is C₆H₃-**Z¹**, wherein **Z¹** is a carbonyl, ester, or amide that is covalently attached to the $-(CH_2)_{q1}-$ moiety;

q is an integer of 1-9; and

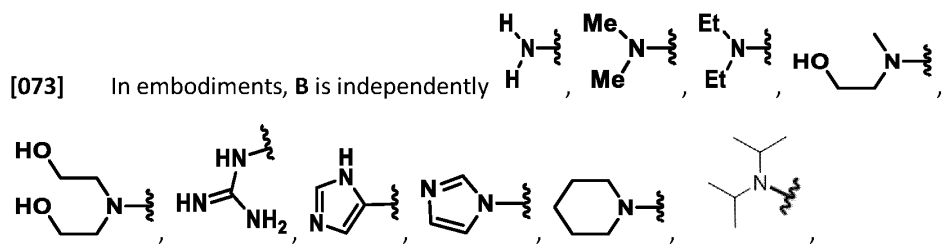
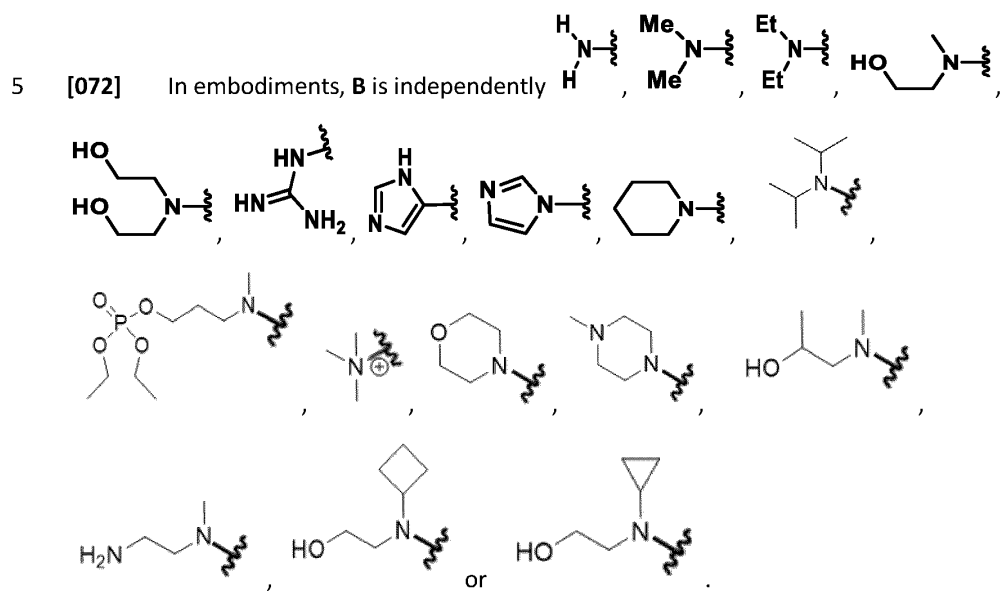
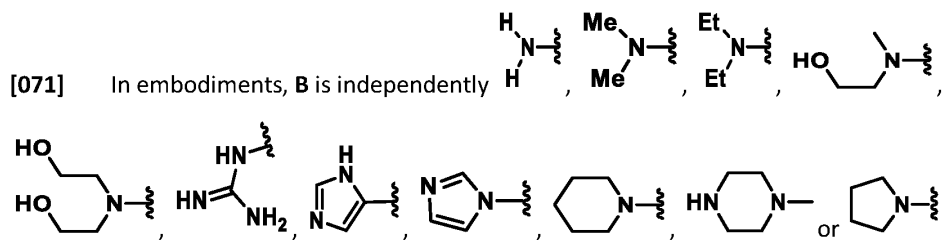
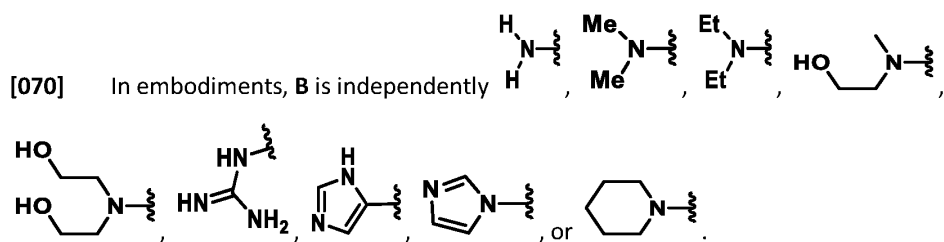
15 **B** is independently an ionizable nitrogen-containing group.

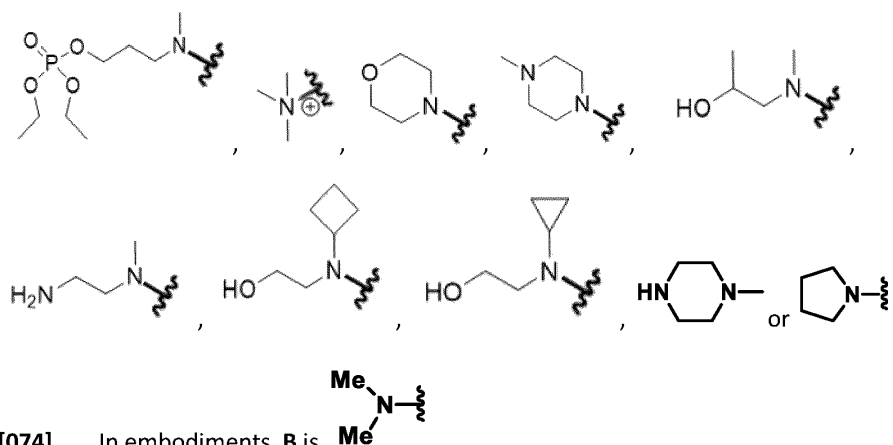
[067] In embodiments, **X** is O; each **L⁴** is $-C(O)O-$; **m** is an integer of 2, 3, or 4; and/or **q** is 1.

[068] In embodiments, **Z** is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, and **q₁** and **q₂** are each 2.



[069] In embodiments, **Z** is .





[075] In embodiments, a compound is Compound (16) or (31).

- 5 [076] In one aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound
- 10 having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof.

- [077] In one aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according
- 15 to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof.

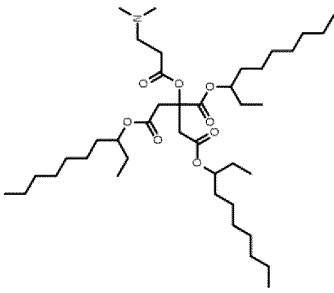
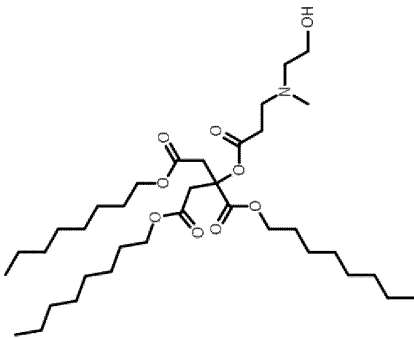
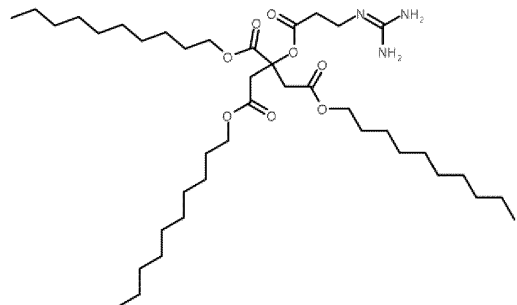
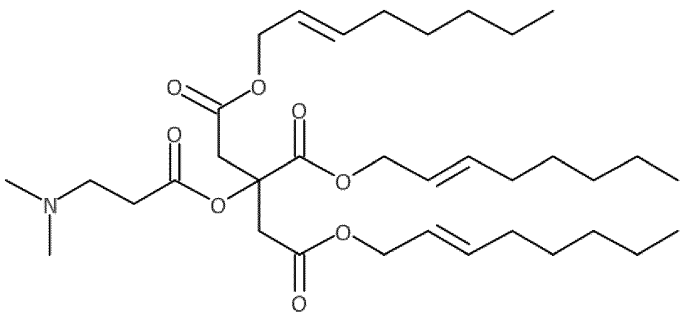
- [078] In one aspect, the invention features a method of intranasal delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids,
- 20 optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof, for example Compound 64, or a compound in Table 6, or a pharmaceutically acceptable salt thereof.

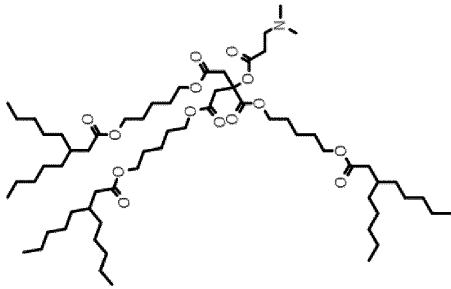
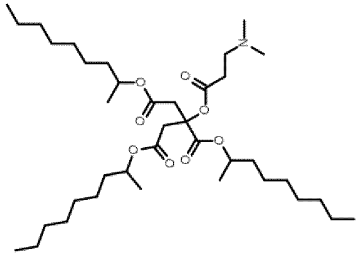
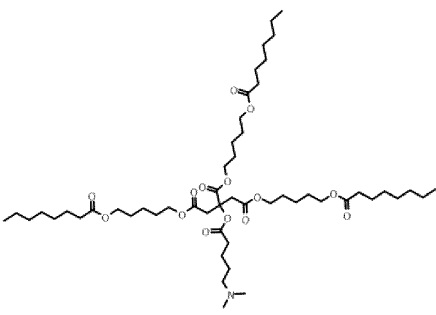
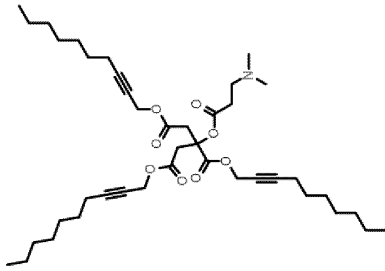
[079] In one aspect, the invention features a method of intranasal delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof, for example

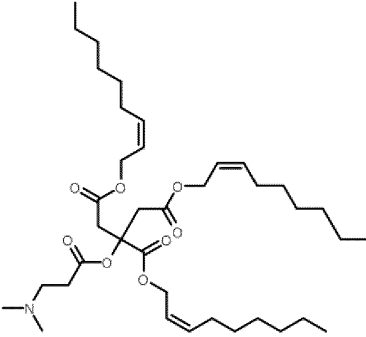
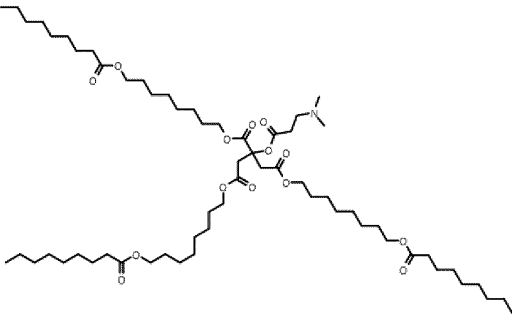
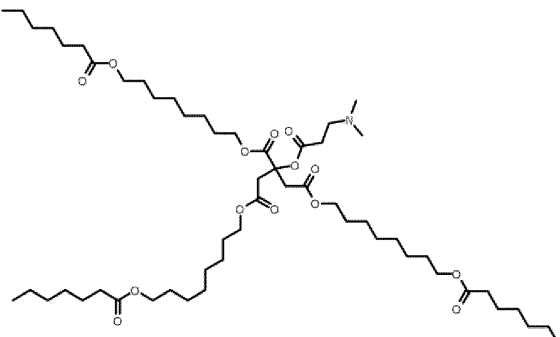
5 Compound 64, or a compound in Table 6, or a pharmaceutically acceptable salt thereof.

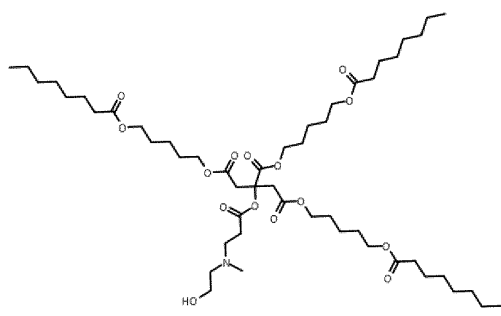
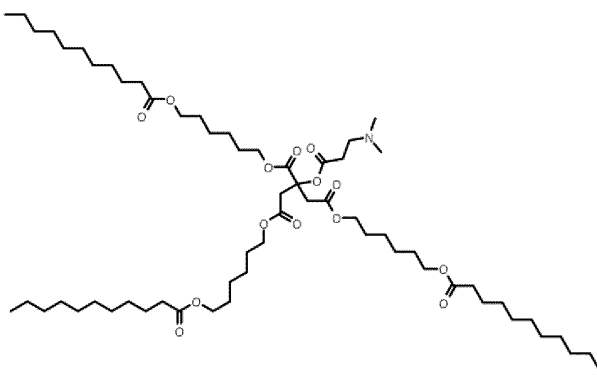
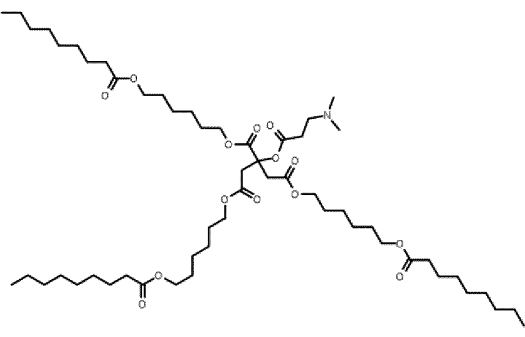
[080] In another aspect, a compound is selected from the group of Compounds 41-64 and 90, or a pharmaceutically acceptable salt thereof:

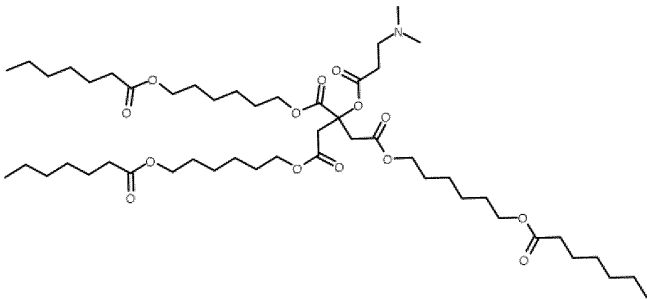
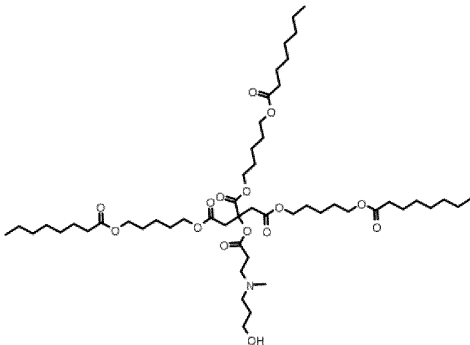
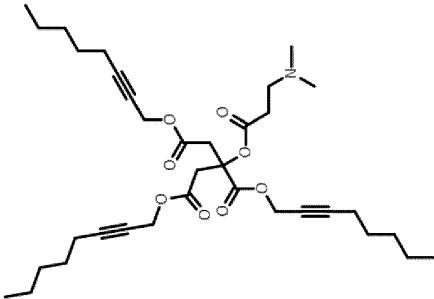
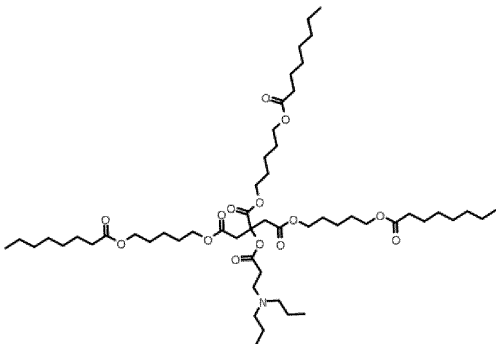
No.	Structure
41	
42	
43	

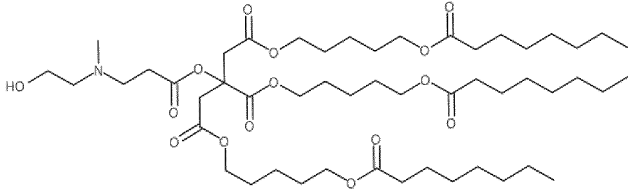
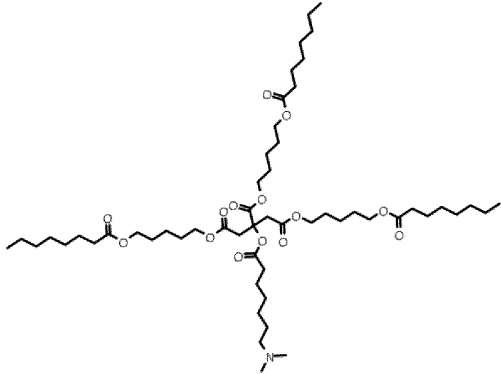
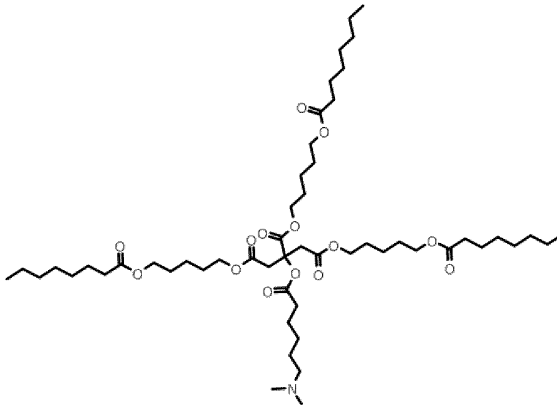
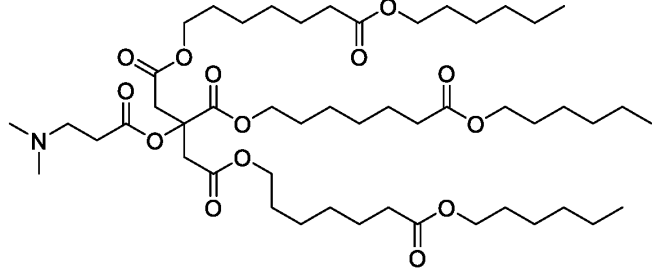
No.	Structure
44	
45	
46	
47	

No.	Structure
48	
49	
50	
51	

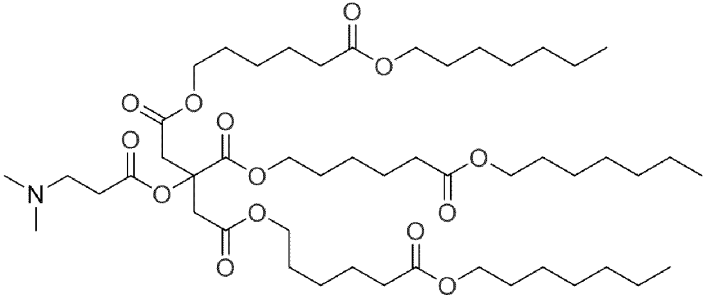
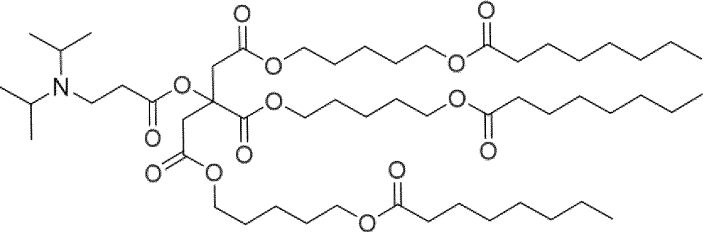
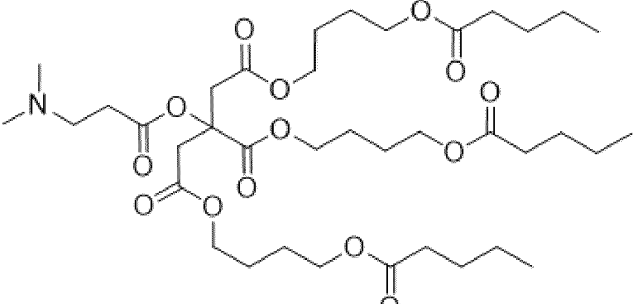
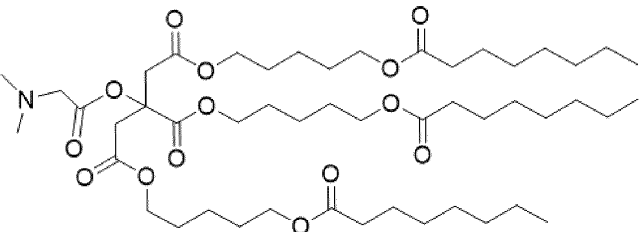
No.	Structure
52	
53	
54	

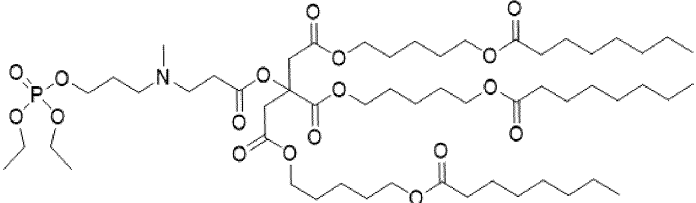
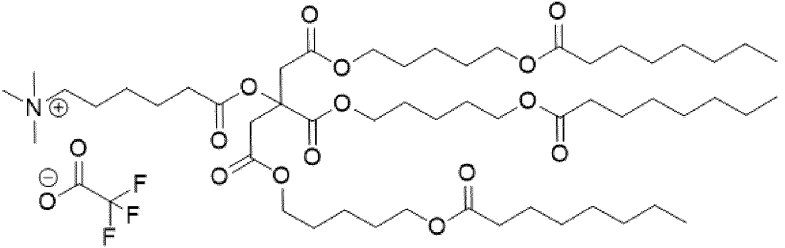
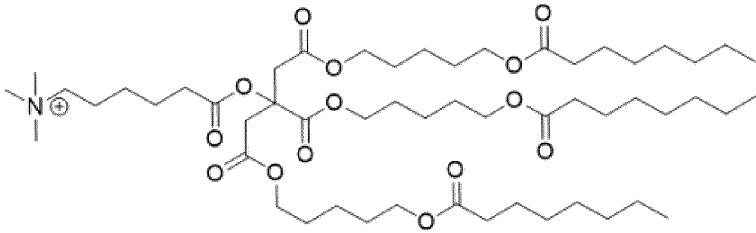
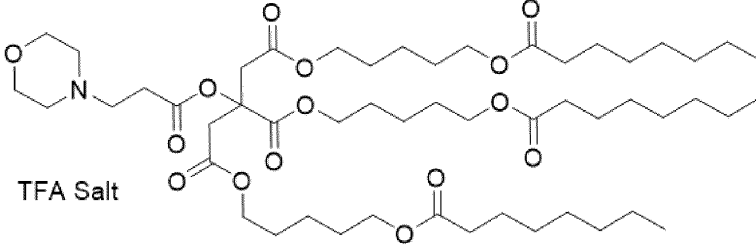
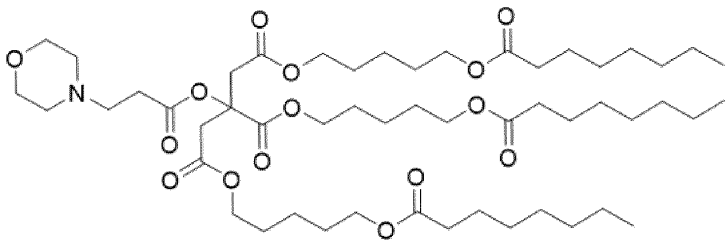
No.	Structure
55	
56	
57	

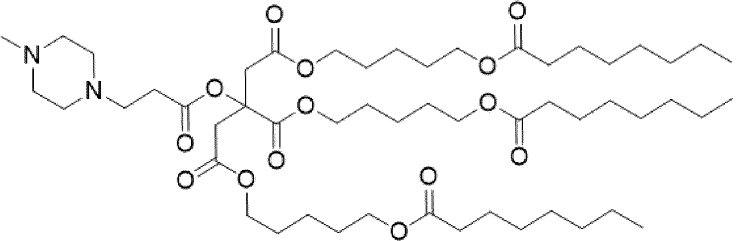
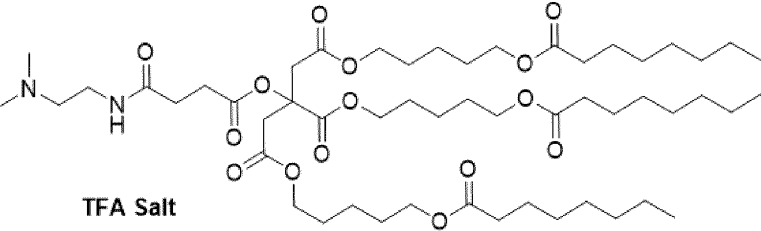
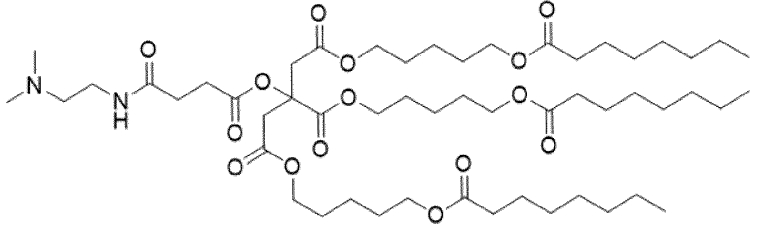
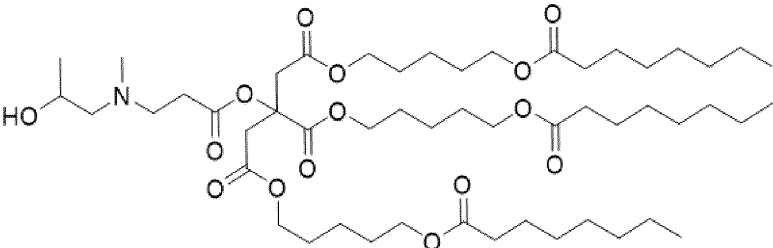
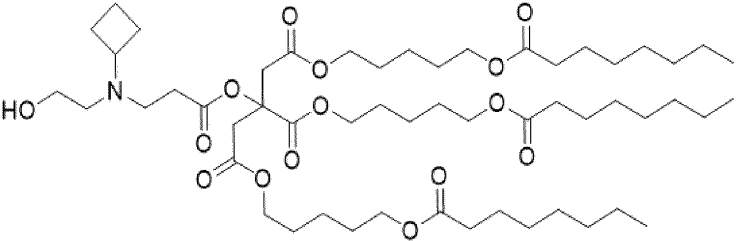
No.	Structure
58	
59	
60	
61	

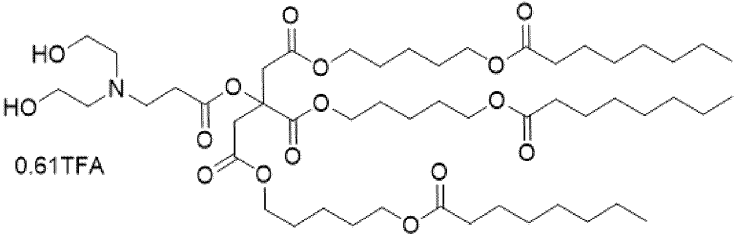
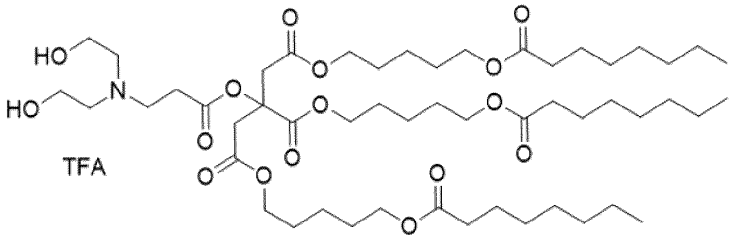
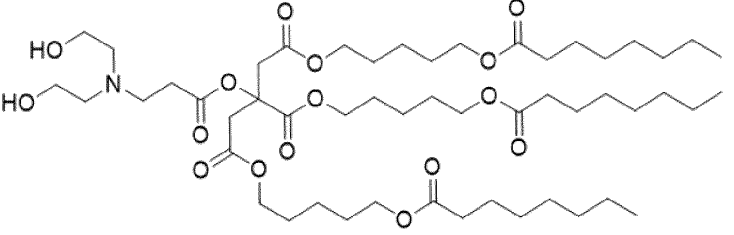
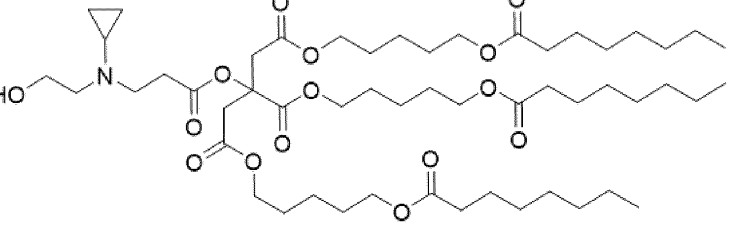
No.	Structure
62	
63	
64	
90	

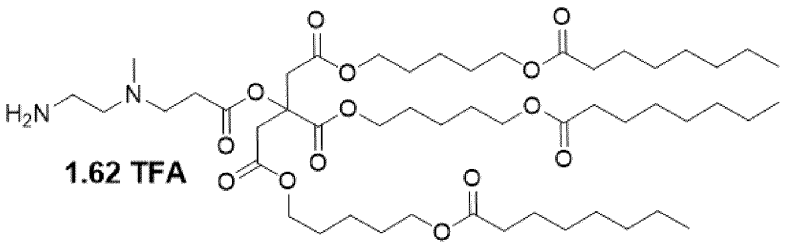
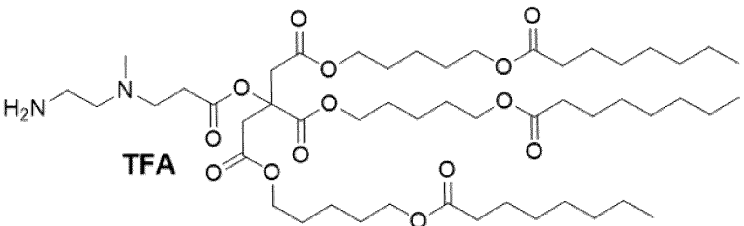
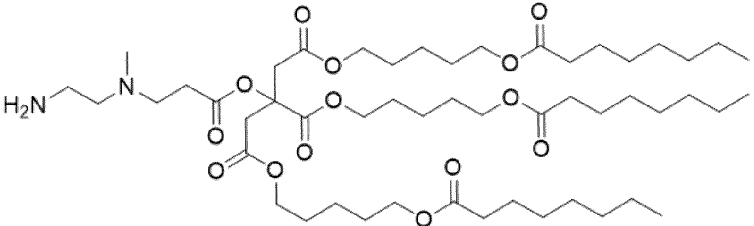
[081] In another aspect, a compound is selected from the group of Compounds 91-111, or a pharmaceutically acceptable salt thereof:

No.	Structure
91	
92	
93	
94	

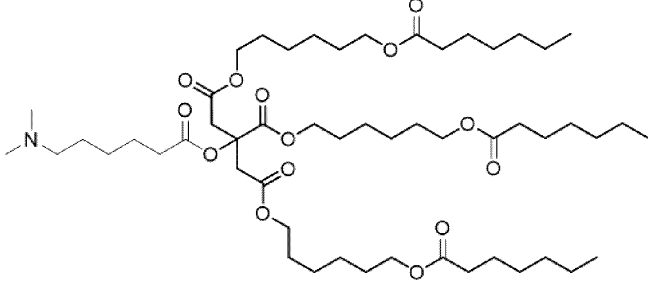
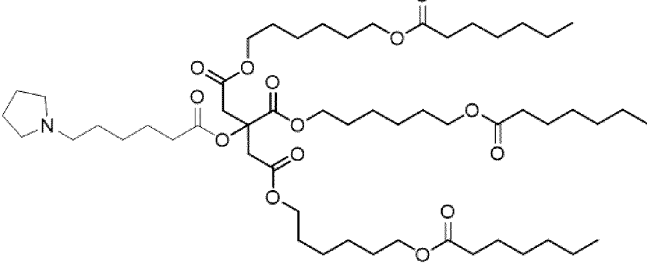
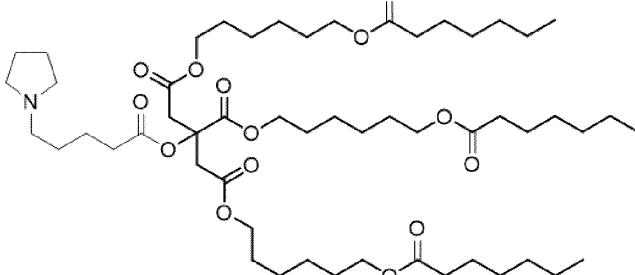
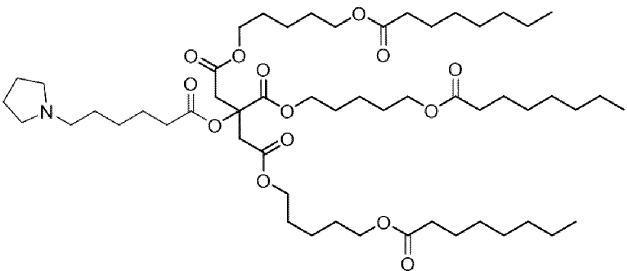
No.	Structure
95	
96	
97	
98	 TFA Salt
99	

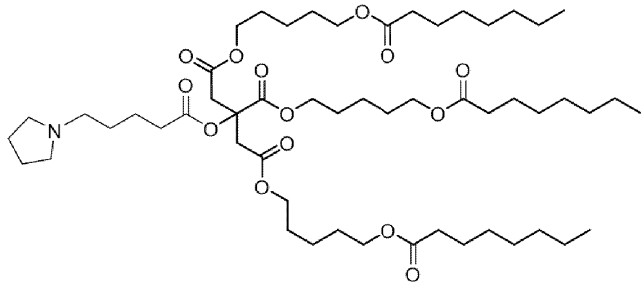
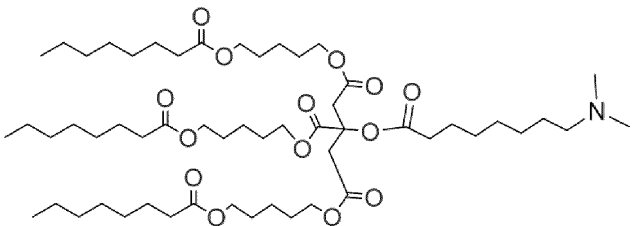
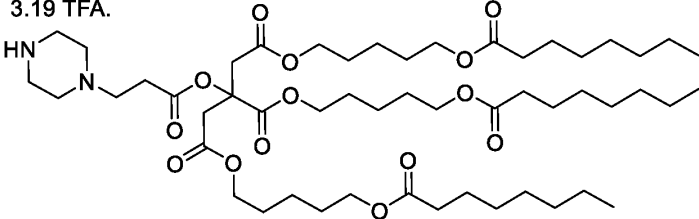
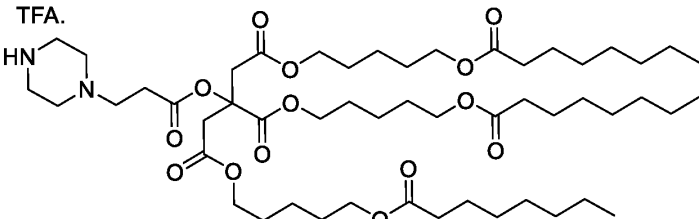
No.	Structure
100	
101	 TFA Salt
102	
103	
104	

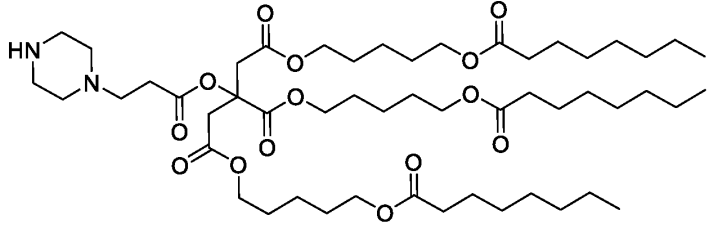
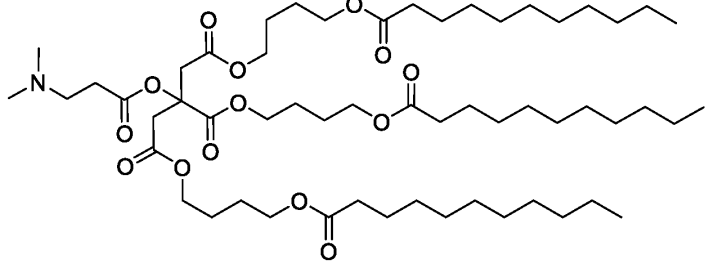
No.	Structure
105	 0.61TFA
106	 TFA
107	
108	

No.	Structure
109	 1.62 TFA TFA content 15% (w/w)
110	 TFA
111	

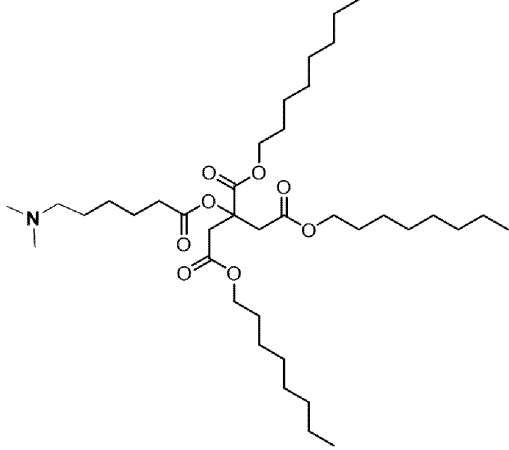
[082] In another aspect, a compound is selected from the group of Compounds 112-117 and 126-129, or a pharmaceutically acceptable salt thereof:

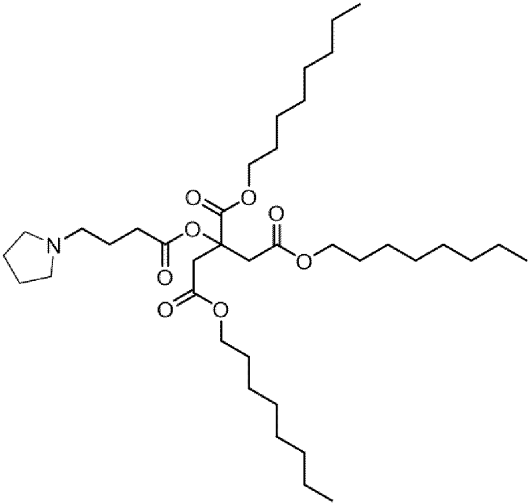
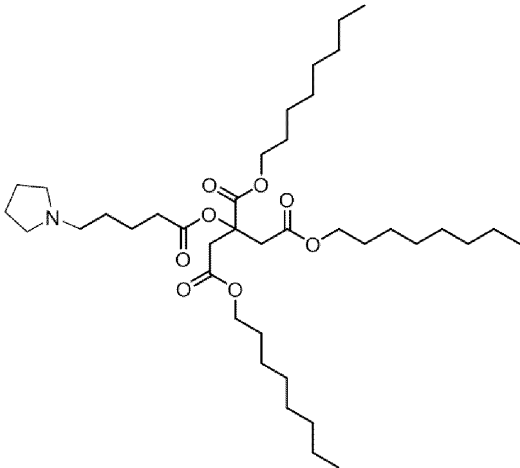
No.	Structure
112	 <chem>CCN(C)CCCCC(=O)O[C@]1(CCCC(=O)OCCCC)C(=O)OCCCCC(=O)OCCCCC(=O)OCCCC</chem>
113	 <chem>C1CCNCC1CCCCC(=O)O[C@]1(CCCC(=O)OCCCC)C(=O)OCCCCC(=O)OCCCCC(=O)OCCCC</chem>
114	 <chem>C1CCNCC1CCCCC(=O)O[C@]1(CCCC(=O)OCCCC)C(=O)OCCCCC(=O)OCCCCC(=O)OCCCC</chem>
115	 <chem>C1CCNCC1CCCCC(=O)O[C@]1(CCCC(=O)OCCCC)C(=O)OCCCCC(=O)OCCCCC(=O)OCCCC</chem>

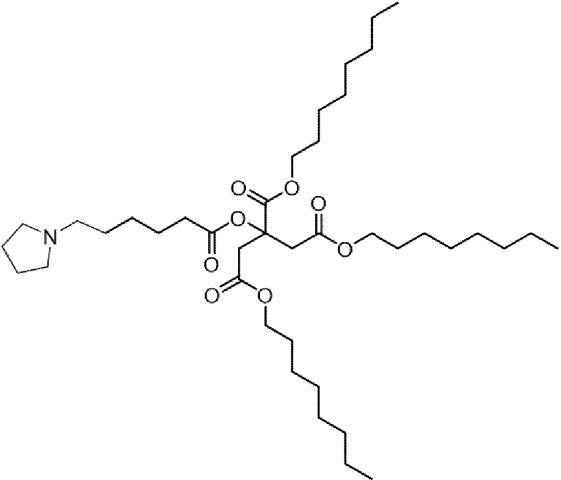
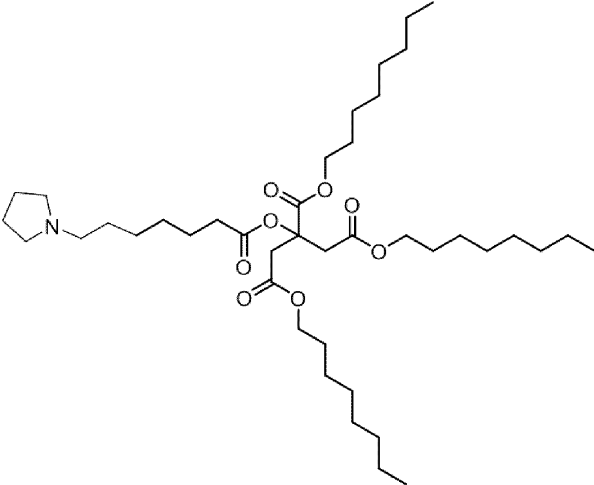
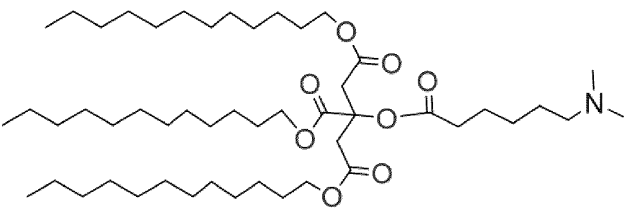
No.	Structure
116	
117	
126	3.19 TFA. 
127	TFA. 

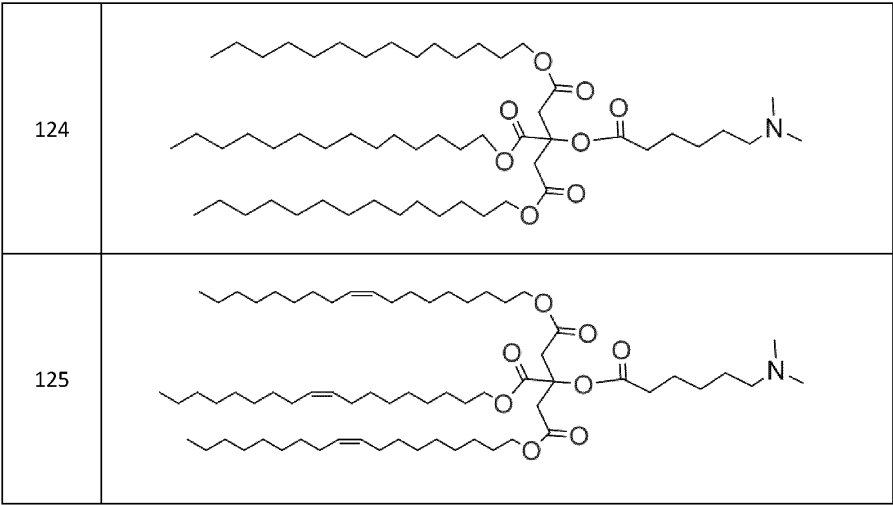
No.	Structure
128	
129	

[083] In another aspect, a compound is selected from the group of Compounds 118-125, or a pharmaceutically acceptable salt thereof:

118	
-----	--

119	
120	

121	 <chem>CCCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCCC)C(=O)OCCCCCCCCC</chem>
122	 <chem>CCCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCCC)C(=O)OCCCCCCCCC</chem>
123	 <chem>CCCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCCC)C(=O)OCCCCCCCCC</chem>



[084] In embodiments, a composition comprises an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is any cationic lipid described herein.

[085] In embodiments, a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is any cationic lipid described herein.

[086] In embodiments, a composition further comprises one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids.

[087] In embodiments, a nucleic acid is an mRNA encoding a peptide or polypeptide.

[088] In embodiments, an mRNA that encodes a peptide or polypeptide for use in vaccine.

[089] In embodiments, an mRNA encodes an antigen.

[090] In embodiments, an antigen is from an infectious agent.

[091] In embodiments, a composition is formulated for a route of administration that is oral, rectal, vaginal, transmucosal, pulmonary, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary

injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, or intranasal.

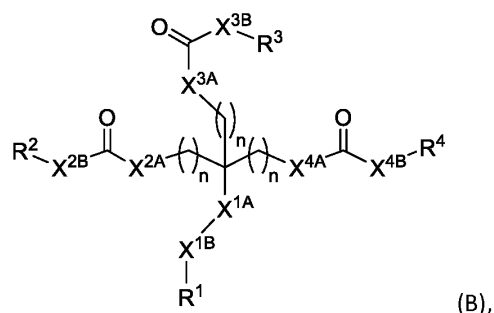
[092] In embodiments, pulmonary delivery is intratracheal or inhaled.

[093] In embodiments, a route of administration is intranasal.

5 [094] In embodiments, a route of administration is intramuscular.

[095] In embodiments, a route of administration is pulmonary.

[096] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B):



15 or a pharmaceutically acceptable salt thereof, wherein
 each n is independently 0 or 1;
 X^{1A} is independently O or NR^{1A} ;
 R^{1A} is H or C_1 - C_6 alkyl;
 X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;
 20 one of X^{2A} and X^{2B} is O and the other is a covalent bond;
 one of X^{3A} and X^{3B} is O and the other is a covalent bond;
 one of X^{4A} and X^{4B} is O and the other is a covalent bond;
 R^1 is independently L^1 - B^1 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 R^2 is independently L^2 - B^2 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 25 R^3 is independently L^3 - B^3 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^4 is independently L^4-B^4 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

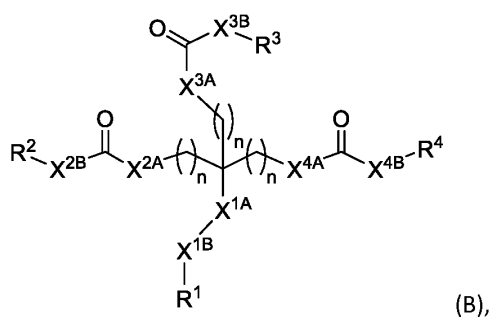
L^1 , L^2 , L^3 , and L^4 are each independently C_1-C_{30} alkylene; C_2-C_{30} alkenylene; or C_2-C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

5 and

wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

[097] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A} ;

15 R^{1A} is H or C_1-C_6 alkyl;

X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

20 R^1 is independently L^1-B^1 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

R^2 is independently L^2-B^2 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

R^3 is independently L^3-B^3 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

R^4 is independently L^4-B^4 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

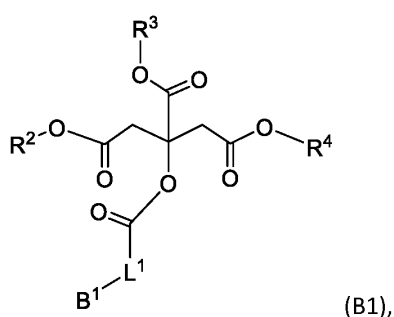
25 L^1 , L^2 , L^3 , and L^4 are each independently C_1-C_{30} alkylene; C_2-C_{30} alkenylene; or C_2-C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

and

wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

- [098] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B1):

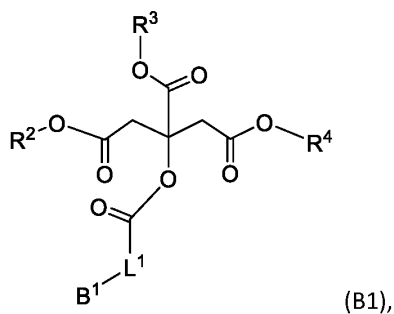


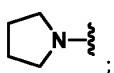
- 10 wherein B¹ is ;

L¹ is straight-chain C₅ alkylene; and

R², R³, and R⁴ are as defined for Formula (B).

- [099] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B1):
- 15

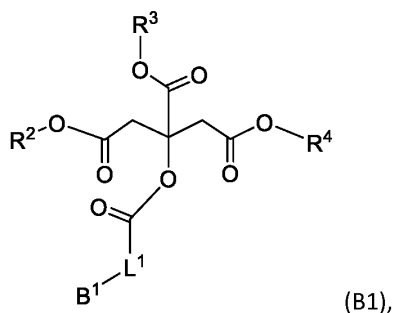


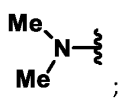
wherein B¹ is  ;

L¹ is as defined for Formula (B); and

R², R³, and R⁴ are straight-chain C₈ alkylene.

- 5 **[0100]** In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B1):

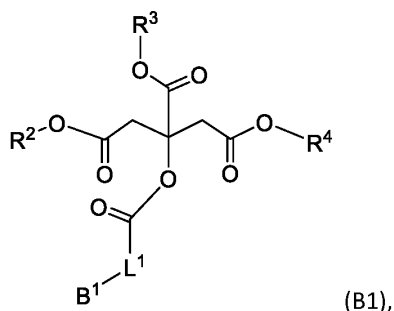


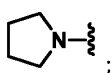
- 10 wherein B¹ is  ;

L¹ is as defined for Formula (B); and

R², R³, and R⁴ are straight-chain C₈ alkylene.

- [0101]** In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome,
- 15 wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B1):



wherein B¹ is  ;

L¹ is as defined for Formula (B); and

R², R³, and R⁴ are straight-chain C₈ alkylene.

- 5 **[0102]** In embodiments the present invention also provides compounds of formula (B1) as described herein. Said compounds may be incorporated into any of the compositions disclosed herein and may be used in any of the methods described herein.

[0103] In embodiments, a composition further comprises one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more

- 10 PEG-modified lipids.

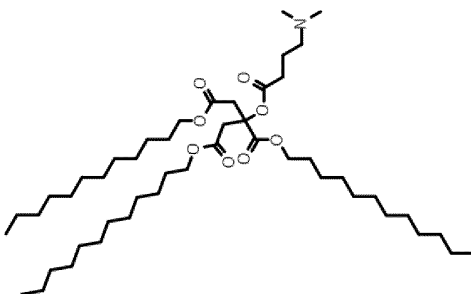
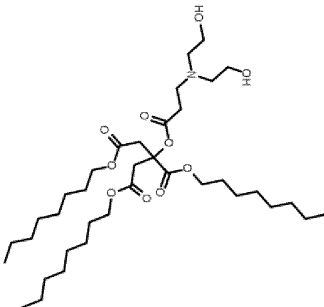
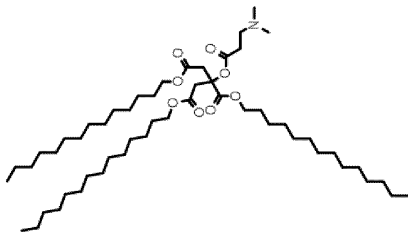
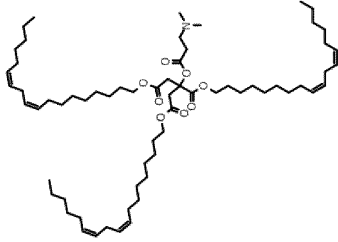
[0104] In embodiments, a nucleic acid is an mRNA encoding a peptide or polypeptide.

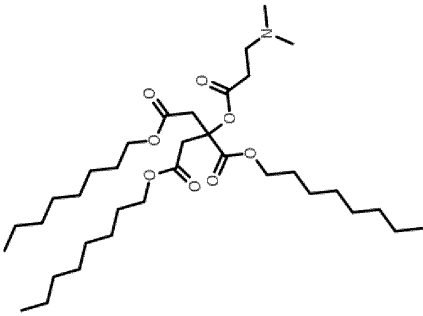
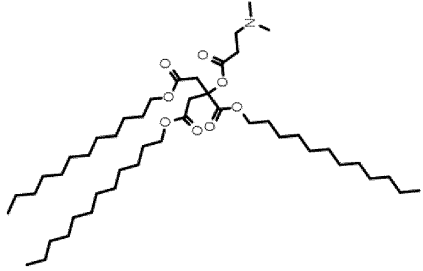
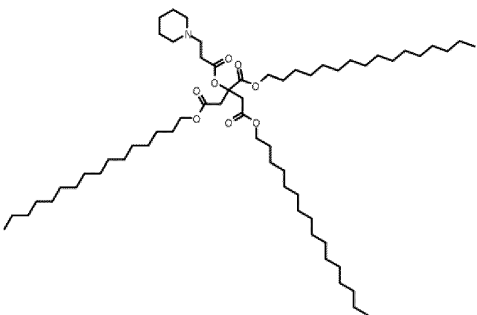
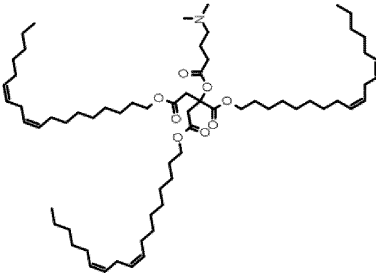
[0105] In embodiments, a composition comprises an mRNA that encodes a peptide or polypeptide for use in vaccine.

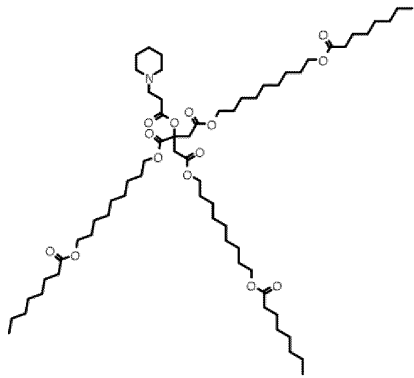
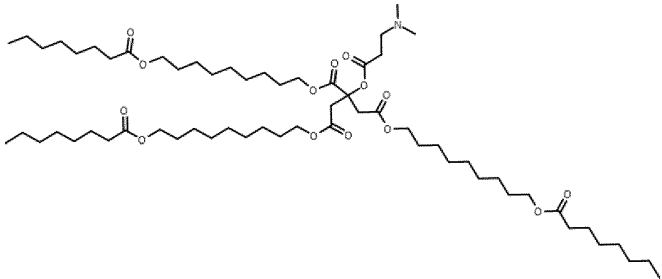
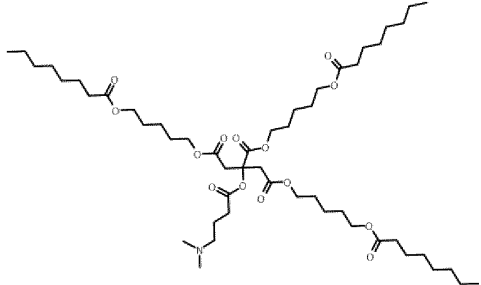
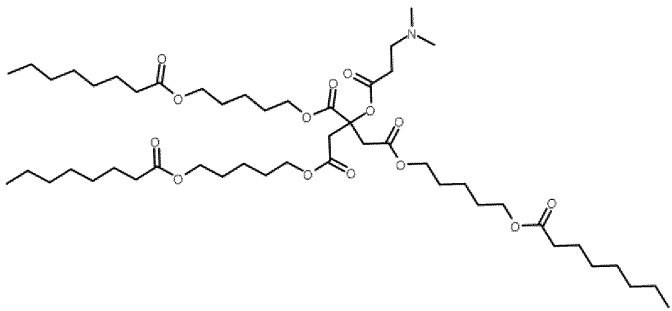
[0106] In embodiments, an mRNA encodes an antigen.

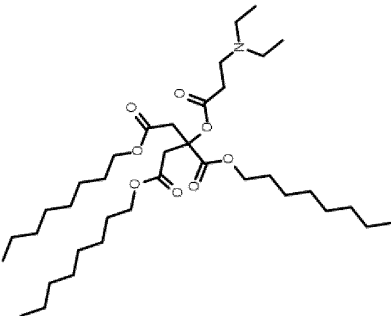
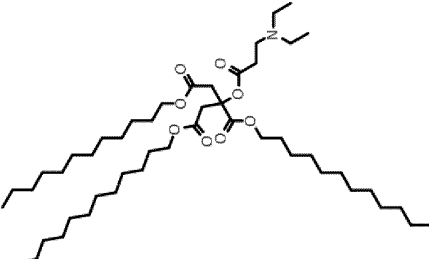
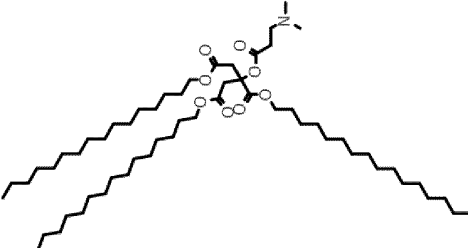
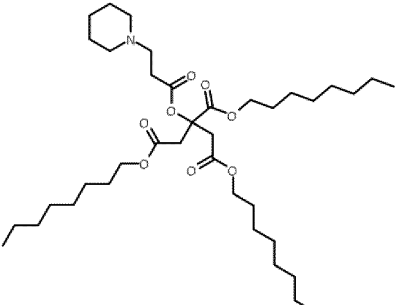
- 15 **[0107]** In embodiments, an antigen is from an infectious agent.

[0108] In embodiments, a compound is selected from the group consisting of Compounds (65)-(89), or a pharmaceutically acceptable salt thereof:

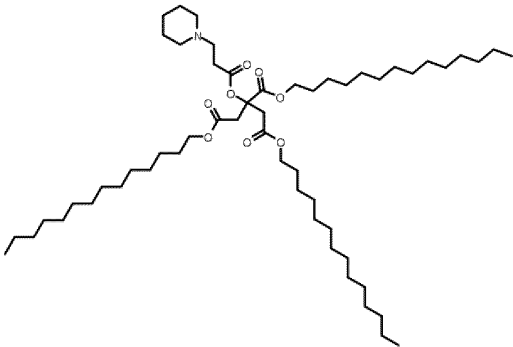
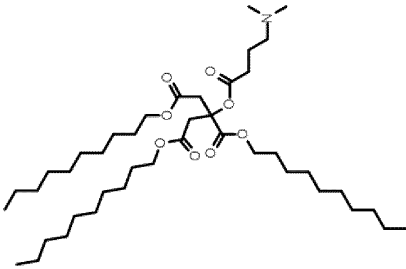
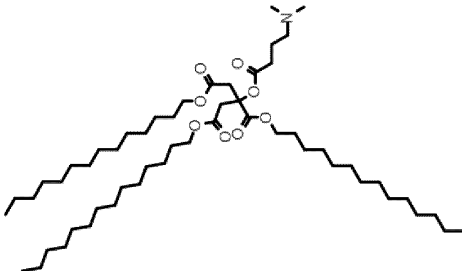
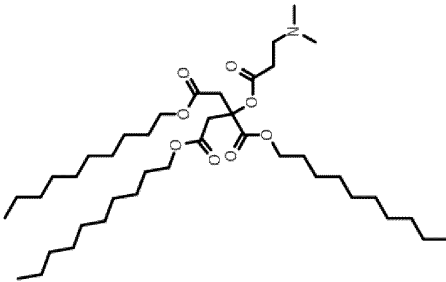
No.	Structure
65	
66	
67	
68	

No.	Structure
69	
70	
71	
72	

No.	Structure
73	 Chemical structure 73: A central carbon atom bonded to a piperidine ring, a long alkyl chain, and two long alkyl chains via ester linkages.
74	 Chemical structure 74: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains via ester linkages.
75	 Chemical structure 75: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains via ester linkages.
76	 Chemical structure 76: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains via ester linkages.

No.	Structure
77	
78	
79	
80	

No.	Structure
81	
82	
83	
84	

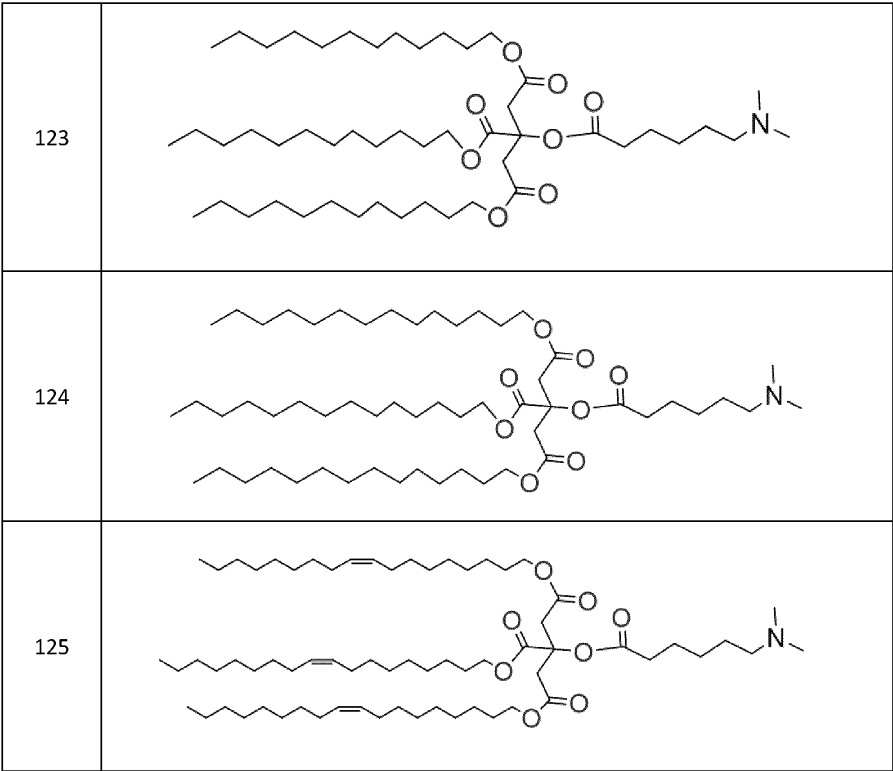
No.	Structure
85	
86	
87	
88	

No.	Structure
89	

[0109] In embodiments, a compound is selected from the group consisting of Compounds (118)-(125), or a pharmaceutically acceptable salt thereof:

118	
119	

120	<chem>CCCCN1CCCC1CCCC(=O)OC(C(=O)OCCCCC)C(=O)OCCCCC</chem>
121	<chem>CCCCN1CCCC1CCCC(=O)OC(C(=O)OCCCCC)C(=O)OCCCCC</chem>
122	<chem>CCCCN1CCCC1CCCC(=O)OC(C(=O)OCCCCC)C(=O)OCCCCC</chem>



[0110] In embodiments, delivery is intranasal.

[0111] In embodiments, delivery is intramuscular.

[0112] In embodiments, delivery is pulmonary.

5

BRIEF DESCRIPTION OF DRAWINGS

[0113] FIG. 1 depicts % survival of BALB/c mice inoculated 1) intranasally (IN) with Lipid Nanoparticles (LNPs) encapsulating CA09 HA mRNA (these LNPs comprised either Compound 64 or the lipid OF-02); 2) intramuscularly (IM) with LNPs encapsulating CA09 HA mRNA (these LNPs comprised OF-02 only); or 3) a phosphate-buffered saline (PBS) buffer control administered intranasally (IN). These results demonstrate high efficacy of LNPs comprising Compound 64 administered intranasally in a lethal mouse influenza challenge model.

[0114] FIG. 2 depicts % body weight change of BALB/c mice inoculated 1) intranasally (IN) with Lipid Nanoparticles (LNPs) encapsulating CA09 HA mRNA (these LNPs comprised either Compound 64 or the lipid OF-02); 2) intramuscularly (IM) with LNPs encapsulating CA09 HA mRNA (these LNPs comprised OF-02 only); or 3) a phosphate-buffered saline (PBS) buffer control administered

15

intranasally (IN). These results demonstrate high efficacy of LNPs comprising Compound 64 administered intranasally in a lethal mouse influenza challenge model. The data presented in the graph of FIG.2 extends to 14 days post-challenge

- [0115] FIG. 3 and FIG. 4 depict data obtained from various studies comparing intranasal administration of Compound 64 (a compound of the invention) with intramuscular administration of known compound OF-02 which are described in more detail in Example 47 herein.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Definitions

- 10 [0116] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.
- 15 [0117] *Amino acid*: As used herein, the term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in
- 20 some embodiments, an amino acid is an l-amino acid. “Standard amino acid” refers to any of the twenty standard l-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid
- 25 derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide’s circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as
- 30 association with one or more chemical entities (*e.g.*, methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, *etc.*). The term “amino acid” is

used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0118] *Animal:* As used herein, the term “animal” refers to any member of the animal kingdom.

- 5 In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, a bovine, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms.
- 10 In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0119] *Approximately or about:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values

15 that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0120] *Biologically active:* As used herein, the term “biologically active” refers to a

20 characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active.

[0121] *Delivery:* As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA encompasses situations in which an mRNA is delivered

25 to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient’s circulation system (*e.g.*, serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery”).

- 30 **[0122]** *Expression:* As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a polypeptide, assemble multiple polypeptides into an intact protein (*e.g.*, enzyme) and/or post-translational modification of a polypeptide or fully assembled protein (*e.g.*,

enzyme). In this application, the terms “expression” and “production,” and grammatical equivalents thereof, are used interchangeably.

[0123] *Functional*: As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

5 [0124] *Half-life*: As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0125] *Helper lipid*: The term “helper lipid” as used herein refers to any neutral or zwitterionic lipid material including cholesterol. Without wishing to be held to a particular theory, helper lipids
10 may add stability, rigidity, and/or fluidity within lipid bilayers/nanoparticles.

[0126] *Improve, increase, or reduce*: As used herein, the terms “improve,” “increase,” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the
15 absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0127] *In Vitro*: As used herein, the term “*in vitro*” refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-
20 cellular organism.

[0128] *In Vivo*: As used herein, the term “*in vivo*” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

25 [0129] *Isolated*: As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about
30 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with

which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (*e.g.*, buffer, solvent, water, *etc.*).

[0130] *Liposome*: As used herein, the term “liposome” refers to any lamellar, multilamellar, or solid nanoparticle vesicle. Typically, a liposome as used herein can be formed by mixing one or more lipids or by mixing one or more lipids and polymer(s). In some embodiments, a liposome suitable for the present invention contains a cationic lipid(s) and optionally non-cationic lipid(s), optionally cholesterol-based lipid(s), and/or optionally PEG-modified lipid(s).

[0131] *messenger RNA (mRNA)*: As used herein, the term “messenger RNA (mRNA)” or “mRNA” refers to a polynucleotide that encodes at least one polypeptide. mRNA as used herein encompasses both modified and unmodified RNA. The term “modified mRNA” related to mRNA comprising at least one chemically modified nucleotide. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, *etc.*

An mRNA sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (*e.g.*, adenosine, guanosine, cytidine, uridine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (*e.g.*, 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5′-*N*-phosphoramidite linkages).

[0132] *Nucleic acid*: As used herein, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a

polynucleotide chain via a phosphodiester linkage. In some embodiments, “nucleic acid” refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some embodiments, “nucleic acid” refers to a polynucleotide chain comprising individual nucleic acid residues. In some embodiments, “nucleic acid” encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. In some embodiments, “nucleic acid” encompasses ribonucleic acids (RNA), including but not limited to any one or more of interference RNAs (RNAi), small interfering RNA (siRNA), short hairpin RNA (shRNA), antisense RNA (aRNA), messenger RNA (mRNA), modified messenger RNA (mmRNA), long non-coding RNA (lncRNA), micro-RNA (miRNA) multimeric coding nucleic acid (MCNA), polymeric coding nucleic acid (PCNA), guide RNA (gRNA) and CRISPR RNA (crRNA). In some embodiments, “nucleic acid” encompasses deoxyribonucleic acid (DNA), including but not limited to any one or more of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) and complementary DNA (cDNA). In some embodiments, “nucleic acid” encompasses both RNA and DNA. In embodiments, DNA may be in the form of antisense DNA, plasmid DNA, parts of a plasmid DNA, pre-condensed DNA, a product of a polymerase chain reaction (PCR), vectors (*e.g.*, P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, chromosomal DNA, or derivatives of these groups. In embodiments, RNA may be in the form of messenger RNA (mRNA), ribosomal RNA (rRNA), signal recognition particle RNA (7 SL RNA or SRP RNA), transfer RNA (tRNA), transfer-messenger RNA (tmRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), SmY RNA, small Cajal body-specific RNA (scaRNA), guide RNA (gRNA), ribonuclease P (RNase P), Y RNA, telomerase RNA component (TERC), spliced leader RNA (SL RNA), antisense RNA (aRNA or asRNA), cis-natural antisense transcript (cis-NAT), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), micro-RNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), transacting siRNA (tasiRNA), repeat associated siRNA (rasiRNA), 73K RNA, retrotransposons, a viral genome, a viroid, satellite RNA, or derivatives of these groups. In some embodiments, a nucleic acid is a mRNA encoding a protein such as an enzyme.

[0133] *Patient:* As used herein, the term “patient” or “subject” refers to any organism to which a provided composition may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre- and post-natal forms.

[0134] *Pharmaceutically acceptable:* The term “pharmaceutically acceptable,” as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in

contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0135] *Pharmaceutically acceptable salt*: Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge *et al.*, describes pharmaceutically acceptable salts in detail in *J.*

5 *Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases.

Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric

10 acid, citric acid, succinic acid, or malonic acid, or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, 15 heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases 20 include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4} \text{ alkyl})_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, sulfonate, and aryl sulfonate. Further 25 pharmaceutically acceptable salts include salts formed from the quaternization of an amine using an appropriate electrophile, *e.g.*, an alkyl halide, to form a quaternized alkylated amino salt.

[0136] *Systemic distribution or delivery*: As used herein, the terms “systemic distribution” or “systemic delivery,” or grammatical equivalents thereof, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic 30 distribution or delivery is accomplished via body’s circulation system, *e.g.*, blood stream. Compared to the definition of “local distribution or delivery.”

[0137] *Subject*: As used herein, the term “subject” refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0138] *Substantially*: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0139] *Target tissues*: As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

[0140] *Therapeutically effective amount*: As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0141] *Treating*: As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0142] *Acyl*: As used herein, the term “acyl” refers to $R^Z-(C=O)-$, wherein R^Z is, for example, any alkyl, alkenyl, alkynyl, heteroalkyl or heteroalkylene.

[0143] *Aliphatic*: As used herein, the term aliphatic refers to C_1 - C_{50} hydrocarbons and includes both saturated and unsaturated hydrocarbons. An aliphatic may be linear, branched, or cyclic.

- 5 For example, C_1 - C_{20} aliphatics can include C_1 - C_{20} alkyls (*e.g.*, linear or branched C_1 - C_{20} saturated alkyls), C_2 - C_{20} alkenyls (*e.g.*, linear or branched C_4 - C_{20} dienyls, linear or branched C_6 - C_{20} trienyls, and the like), and C_2 - C_{20} alkynyls (*e.g.*, linear or branched C_2 - C_{20} alkynyls). C_1 - C_{20} aliphatics can include C_3 - C_{20} cyclic aliphatics (*e.g.*, C_3 - C_{20} cycloalkyls, C_4 - C_{20} cycloalkenyls, or C_8 - C_{20} cycloalkynyls). In certain embodiments, the aliphatic may comprise one or more cyclic aliphatic and/or one or
- 10 more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxy, hydroxy, amino, aryl, ether, ester or amide. An aliphatic group is unsubstituted or substituted with one or more substituent groups as described herein. For example, an aliphatic may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, $-COR''$, $-CO_2H$, $-CO_2R''$, $-CN$, $-OH$, $-OR''$, $-OCOR'$, $-$
- 15 OCO_2R'' , $-NH_2$, $-NHR''$, $-N(R'')_2$, $-SR''$ or $-SO_2R''$, wherein each instance of R'' independently is C_1 - C_{20} aliphatic (*e.g.*, C_1 - C_{20} alkyl, C_1 - C_{15} alkyl, C_1 - C_{10} alkyl, or C_1 - C_3 alkyl). In embodiments, R'' independently is an unsubstituted alkyl (*e.g.*, unsubstituted C_1 - C_{20} alkyl, C_1 - C_{15} alkyl, C_1 - C_{10} alkyl, or C_1 - C_3 alkyl). In embodiments, R'' independently is unsubstituted C_1 - C_3 alkyl. In embodiments, the aliphatic is unsubstituted. In embodiments, the aliphatic does not include any heteroatoms.
- 20 *Alkyl*: As used herein, the term “alkyl” means acyclic linear and branched hydrocarbon groups, *e.g.* “ C_1 - C_{30} alkyl” refers to alkyl groups having 1-30 carbons. An alkyl group may be linear or branched. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, hexyl, isohexyl, *etc.* The term “lower alkyl” means an alkyl group straight chain or branched alkyl having 1 to 6 carbon
- 25 atoms. Other alkyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. An alkyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkyl group may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, $-COR''$, $-CO_2H$, $-$
- 30 CO_2R'' , $-CN$, $-OH$, $-OR''$, $-OCOR'$, $-OCO_2R''$, $-NH_2$, $-NHR''$, $-N(R'')_2$, $-SR''$ or $-SO_2R''$, wherein each instance of R'' independently is C_1 - C_{20} aliphatic (*e.g.*, C_1 - C_{20} alkyl, C_1 - C_{15} alkyl, C_1 - C_{10} alkyl, or C_1 - C_3 alkyl). In embodiments, R'' independently is an unsubstituted alkyl (*e.g.*, unsubstituted C_1 - C_{20} alkyl, C_1 - C_{15} alkyl, C_1 - C_{10} alkyl, or C_1 - C_3 alkyl). In embodiments, R'' independently is unsubstituted C_1 - C_3 alkyl. In embodiments, the alkyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent

groups as described herein). In embodiments, an alkyl group is substituted with a—OH group and may also be referred to herein as a “hydroxyalkyl” group, where the prefix denotes the —OH group and “alkyl” is as described herein.

[0144] As used herein, “alkyl” also refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 50 carbon atoms (“C₁-C₅₀ alkyl”). In some embodiments, an alkyl group has 1 to 40 carbon atoms (“C₁-C₄₀ alkyl”). In some embodiments, an alkyl group has 1 to 30 carbon atoms (“C₁-C₃₀ alkyl”). In some embodiments, an alkyl group has 1 to 20 carbon atoms (“C₁-C₂₀ alkyl”). In some embodiments, an alkyl group has 1 to 10 carbon atoms (“C₁-C₁₀ alkyl”). In some embodiments, an alkyl group has 1 to 9 carbon atoms (“C₁-C₉ alkyl”). In some embodiments, an alkyl group has 1 to 8 carbon atoms (“C₁-C₈ alkyl”). In some embodiments, an alkyl group has 1 to 7 carbon atoms (“C₁-C₇ alkyl”). In some embodiments, an alkyl group has 1 to 6 carbon atoms (“C₁-C₆ alkyl”). In some embodiments, an alkyl group has 1 to 5 carbon atoms (“C₁-C₅ alkyl”). In some embodiments, an alkyl group has 1 to 4 carbon atoms (“C₁-C₄ alkyl”). In some embodiments, an alkyl group has 1 to 3 carbon atoms (“C₁-C₃ alkyl”). In some embodiments, an alkyl group has 1 to 2 carbon atoms (“C₁-C₂ alkyl”). In some embodiments, an alkyl group has 1 carbon atom (“C₁ alkyl”). In some embodiments, an alkyl group has 2 to 6 carbon atoms (“C₂-C₆ alkyl”). Examples of C₁-C₆ alkyl groups include, without limitation, methyl (C₁), ethyl (C₂), n-propyl (C₃), isopropyl (C₃), n-butyl (C₄), tert-butyl (C₄), sec-butyl (C₄), iso-butyl (C₄), n-pentyl (C₅), 3-pentanyl (C₅), amyl (C₅), neopentyl (C₅), 3-methyl-2-butanyl (C₅), tertiary amyl (C₅), and n-hexyl (C₆). Additional examples of alkyl groups include n-heptyl (C₇), n-octyl (C₈) and the like. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an “unsubstituted alkyl”) or substituted (a “substituted alkyl”) with one or more substituents. In certain embodiments, the alkyl group is an unsubstituted C₁-C₅₀ alkyl. In certain embodiments, the alkyl group is a substituted C₁-C₅₀ alkyl.

[0145] Affixing the suffix “-ene” to a group indicates the group is a divalent moiety, *e.g.*, arylene is the divalent moiety of aryl, and heteroarylene is the divalent moiety of heteroaryl.

[0146] *Alkylene*: The term “alkylene,” as used herein, represents a saturated divalent straight or branched chain hydrocarbon group and is exemplified by methylene, ethylene, isopropylene and the like. Likewise, the term “alkenylene” as used herein represents an unsaturated divalent straight or branched chain hydrocarbon group having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, and the term “alkynylene” herein represents an unsaturated divalent straight or branched chain hydrocarbon group having

one or more unsaturated carbon-carbon triple bonds that may occur in any stable point along the chain. In certain embodiments, an alkylene, alkenylene, or alkynylene group may comprise one or more cyclic aliphatic and/or one or more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxy, hydroxy, amino, aryl, ether, ester or amide. For example, an alkylene, alkenylene, or alkynylene may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR'', -CO₂H, -CO₂R'', -CN, -OH, -OR'', -OCOR'', -OCO₂R'', -NH₂, -NHR'', -N(R'')₂, -SR'' or -SO₂R'', wherein each instance of R'' independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is unsubstituted C₁-C₃ alkyl. In certain embodiments, an alkylene, alkenylene, or alkynylene is unsubstituted. In certain embodiments, an alkylene, alkenylene, or alkynylene does not include any heteroatoms. *Alkenyl*: As used herein, "alkenyl" means any linear or branched hydrocarbon chains having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, *e.g.* "C₂-C₃₀ alkenyl" refers to an alkenyl group having 2-30 carbons. For example, an alkenyl group includes prop-2-enyl, but-2-enyl, but-3-enyl, 2-methylprop-2-enyl, hex-2-enyl, hex-5-enyl, 2,3-dimethylbut-2-enyl, and the like. In embodiments, the alkenyl comprises 1, 2, or 3 carbon-carbon double bond. In embodiments, the alkenyl comprises a single carbon-carbon double bond. In embodiments, multiple double bonds (*e.g.*, 2 or 3) are conjugated. An alkenyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkenyl group may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR'', -CO₂H, -CO₂R'', -CN, -OH, -OR'', -OCOR'', -OCO₂R'', -NH₂, -NHR'', -N(R'')₂, -SR'' or -SO₂R'', wherein each instance of R'' independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is unsubstituted C₁-C₃ alkyl. In embodiments, the alkenyl is unsubstituted. In embodiments, the alkenyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent groups as described herein). In embodiments, an alkenyl group is substituted with a-OH group and may also be referred to herein as a "hydroxyalkenyl" group, where the prefix denotes the -OH group and "alkenyl" is as described herein.

[0147] As used herein, "alkenyl" also refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 50 carbon atoms and one or more carbon-carbon double

bonds (e.g., 1, 2, 3, or 4 double bonds) ("C₂-C₅₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 40 carbon atoms ("C₂-C₄₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 30 carbon atoms ("C₂-C₃₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 20 carbon atoms ("C₂-C₂₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 10 carbon atoms ("C₂-C₁₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C₂-C₉ alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C₂-C₈ alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C₂-C₇ alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C₂-C₆ alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C₂-C₅ alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C₂-C₄ alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C₂-C₃ alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C₂ alkenyl"). The one or more carbon-carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of C₂-C₄ alkenyl groups include, without limitation, ethenyl (C₂), 1-propenyl (C₃), 2-propenyl (C₃), 1-butenyl (C₄), 2-butenyl (C₄), butadienyl (C₄), and the like. Examples of C₂-C₆ alkenyl groups include the aforementioned C₂-C₄ alkenyl groups as well as pentenyl (C₅), pentadienyl (C₅), hexenyl (C₆), and the like. Additional examples of alkenyl include heptenyl (C₇), octenyl (C₈), octatrienyl (C₈), and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents. In certain embodiments, the alkenyl group is an unsubstituted C₂-C₅₀ alkenyl. In certain embodiments, the alkenyl group is a substituted C₂-C₅₀ alkenyl.

[0148] *Alkynyl*: As used herein, "alkynyl" means any hydrocarbon chain of either linear or branched configuration, having one or more carbon-carbon triple bonds occurring in any stable point along the chain, e.g., "C₂-C₃₀ alkynyl", refers to an alkynyl group having 2-30 carbons. Examples of an alkynyl group include prop-2-ynyl, but-2-ynyl, but-3-ynyl, pent-2-ynyl, 3-methylpent-4-ynyl, hex-2-ynyl, hex-5-ynyl, etc. In embodiments, an alkynyl comprises one carbon-carbon triple bond. An alkynyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkynyl group may be substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR'', -CO₂H, -CO₂R'', -CN, -OH, -OR'', -OCOR'', -OCO₂R'', -NH₂, -NHR'', -N(R'')₂, -SR'' or -SO₂R'', wherein each instance of R'' independently is C₁-C₂₀ aliphatic (e.g., C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is an unsubstituted alkyl (e.g., unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is

unsubstituted C₁-C₃ alkyl. In embodiments, the alkynyl is unsubstituted. In embodiments, the alkynyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent groups as described herein).

[0149] As used herein, “alkynyl” also refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 50 carbon atoms and one or more carbon-carbon triple bonds (*e.g.*, 1, 2, 3, or 4 triple bonds) and optionally one or more double bonds (*e.g.*, 1, 2, 3, or 4 double bonds) (“C₂-C₅₀ alkynyl”). An alkynyl group that has one or more triple bonds and one or more double bonds is also referred to as an “ene-yne”. In some embodiments, an alkynyl group has 2 to 40 carbon atoms (“C₂-C₄₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 30 carbon atoms (“C₂-C₃₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 20 carbon atoms (“C₂-C₂₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 10 carbon atoms (“C₂-C₁₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 9 carbon atoms (“C₂-C₉ alkynyl”). In some embodiments, an alkynyl group has 2 to 8 carbon atoms (“C₂-C₈ alkynyl”). In some embodiments, an alkynyl group has 2 to 7 carbon atoms (“C₂-C₇ alkynyl”). In some embodiments, an alkynyl group has 2 to 6 carbon atoms (“C₂-C₆ alkynyl”). In some embodiments, an alkynyl group has 2 to 5 carbon atoms (“C₂-C₅ alkynyl”). In some embodiments, an alkynyl group has 2 to 4 carbon atoms (“C₂-C₄ alkynyl”). In some embodiments, an alkynyl group has 2 to 3 carbon atoms (“C₂-C₃ alkynyl”). In some embodiments, an alkynyl group has 2 carbon atoms (“C₂ alkynyl”). The one or more carbon-carbon triple bonds can be internal (such as in 2-butyne) or terminal (such as in 1-butyne). Examples of C₂-C₄ alkynyl groups include, without limitation, ethynyl (C₂), 1-propynyl (C₃), 2-propynyl (C₃), 1-butyne (C₄), 2-butyne (C₄), and the like. Examples of C₂-C₆ alkynyl groups include the aforementioned C₂-C₄ alkynyl groups as well as pentynyl (C₅), hexynyl (C₆), and the like. Additional examples of alkynyl include heptynyl (C₇), octynyl (C₈), and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an “unsubstituted alkynyl”) or substituted (a “substituted alkynyl”) with one or more substituents. In certain embodiments, the alkynyl group is an unsubstituted C₂-C₅₀ alkynyl. In certain embodiments, the alkynyl group is a substituted C₂-C₅₀ alkynyl.

[0150] *Aryl*: The term “aryl” used alone or as part of a larger moiety as in “aralkyl,” refers to a monocyclic, bicyclic, or tricyclic carbocyclic ring system having a total of six to fourteen ring members, wherein said ring system has a single point of attachment to the rest of the molecule, at least one ring in the system is aromatic and wherein each ring in the system contains 4 to 7 ring members. In embodiments, an aryl group has 6 ring carbon atoms (“C₆ aryl,” *e.g.*, phenyl). In some embodiments, an aryl group has 10 ring carbon atoms (“C₁₀ aryl,” *e.g.*, naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms (“C₁₄

aryl," *e.g.*, anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Exemplary aryls include phenyl,
5 naphthyl, and anthracene.

[0151] As used herein, "aryl" also refers to a radical of a monocyclic or polycyclic (*e.g.*, bicyclic or tricyclic) $4n+2$ aromatic ring system (*e.g.*, having 6, 10, or 14 π electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C₆-C₁₄ aryl"). In some embodiments, an aryl group has 6 ring carbon atoms ("C₆ aryl"; *e.g.*,
10 phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("C₁₀ aryl"; *e.g.*, naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("C₁₄ aryl"; *e.g.*, anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms
15 continue to designate the number of carbon atoms in the aryl ring system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents. In certain embodiments, the aryl group is an unsubstituted C₆-C₁₄ aryl. In certain embodiments, the aryl group is a substituted C₆-C₁₄ aryl.

20 **[0152]** *Arylene*: The term "arylene" as used herein refers to an aryl group that is divalent (that is, having two points of attachment to the molecule). Exemplary arylenes include phenylene (*e.g.*, unsubstituted phenylene or substituted phenylene).

[0153] *Carbocyclyl*: As used herein, "carbocyclyl" or "carbocyclic" refers to a radical of a non-aromatic cyclic hydrocarbon group having from 3 to 10 ring carbon atoms ("C₃-C₁₀ carbocyclyl")
25 and zero heteroatoms in the non-aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("C₃-C₈ carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 7 ring carbon atoms ("C₃-C₇ carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C₃-C₆ carbocyclyl"). In some embodiments, a carbocyclyl group has 4 to 6 ring carbon atoms ("C₄-C₆ carbocyclyl"). In some embodiments, a carbocyclyl
30 group has 5 to 6 ring carbon atoms ("C₅-C₆ carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("C₅-C₁₀ carbocyclyl"). Exemplary C₃-C₆ carbocyclyl groups include, without limitation, cyclopropyl (C₃), cyclopropenyl (C₃), cyclobutyl (C₄), cyclobutenyl

(C₄), cyclopentyl (C₅), cyclopentenyl (C₅), cyclohexyl (C₆), cyclohexenyl (C₆), cyclohexadienyl (C₆), and the like. Exemplary C₃-C₈ carbocyclyl groups include, without limitation, the aforementioned C₃-C₆ carbocyclyl groups as well as cycloheptyl (C₇), cycloheptenyl (C₇), cycloheptadienyl (C₇), cycloheptatrienyl (C₇), cyclooctyl (C₈), cyclooctenyl (C₈), bicyclo[2.2.1]heptanyl (C₇), bicyclo[2.2.2]octanyl (C₈), and the like. Exemplary C₃-C₁₀ carbocyclyl groups include, without limitation, the aforementioned C₃-C₈ carbocyclyl groups as well as cyclononyl (C₉), cyclononenyl (C₉), cyclodecyl (C₁₀), cyclodecenyl (C₁₀), octahydro-1H-indenyl (C₉), decahydronaphthalenyl (C₁₀), spiro[4.5]decanyl (C₁₀), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic (“monocyclic carbocyclyl”) or polycyclic (e.g., containing a fused, bridged or spiro ring system such as a bicyclic system (“bicyclic carbocyclyl”) or tricyclic system (“tricyclic carbocyclyl”)) and can be saturated or can contain one or more carbon-carbon double or triple bonds. “Carbocyclyl” also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless otherwise specified, each instance of a carbocyclyl group is independently unsubstituted (an “unsubstituted carbocyclyl”) or substituted (a “substituted carbocyclyl”) with one or more substituents. In certain embodiments, the carbocyclyl group is an unsubstituted C₃-C₁₀ carbocyclyl. In certain embodiments, the carbocyclyl group is a substituted C₃-C₁₀ carbocyclyl.

[0154] In some embodiments, “carbocyclyl” or “carbocyclic” is referred to as a “cycloalkyl”, i.e., a monocyclic, saturated carbocyclyl group having from 3 to 10 ring carbon atoms (“C₃-C₁₀ cycloalkyl”). In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms (“C₃-C₈ cycloalkyl”). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms (“C₃-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 4 to 6 ring carbon atoms (“C₄-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms (“C₅-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms (“C₅-C₁₀ cycloalkyl”). Examples of C₅-C₆ cycloalkyl groups include cyclopentyl (C₅) and cyclohexyl (C₆). Examples of C₃-C₆ cycloalkyl groups include the aforementioned C₅-C₆ cycloalkyl groups as well as cyclopropyl (C₃) and cyclobutyl (C₄). Examples of C₃-C₈ cycloalkyl groups include the aforementioned C₃-C₆ cycloalkyl groups as well as cycloheptyl (C₇) and cyclooctyl (C₈). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted (an “unsubstituted cycloalkyl”) or substituted (a “substituted cycloalkyl”) with one or more

substituents. In certain embodiments, the cycloalkyl group is an unsubstituted C₃-C₁₀ cycloalkyl. In certain embodiments, the cycloalkyl group is a substituted C₃-C₁₀ cycloalkyl.

[0155] *Halogen*: As used herein, the term “halogen” means fluorine, chlorine, bromine, or iodine.

5 **[0156]** *Heteroalkyl*: The term “heteroalkyl” is meant a branched or unbranched alkyl, alkenyl, or alkynyl group having from 4 to 50 carbon atoms in addition to 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O, S, and P. In embodiments, In
embodiments, a heteroalkyl group has 4 to 40 carbon atoms (“C₄-C₄₀ heteroalkyl”). In some
embodiments, a heteroalkyl group has 6 to 30 carbon atoms (“C₆-C₃₀ heteroalkyl”). In some
10 embodiments, a heteroalkyl group has 4 to 20 carbon atoms (“C₄-C₂₀ heteroalkyl”). Heteroalkyls include tertiary amines, secondary amines, ethers, esters, thioethers, amides, thioamides, carbamates, thiocarbamates, hydrazones, imines, phosphodiesteres, phosphoramidates, sulfonamides, and disulfides. A heteroalkyl group may optionally include monocyclic, bicyclic, or tricyclic rings, in which each ring desirably has three to six members. Examples of heteroalkyls
15 include polyethers, such as methoxymethyl and ethoxyethyl. In embodiments, a heteroalkyl comprises an alkyl group as described herein (e.g., a C₄-C₅₀ alkyl or C₆-C₃₀ alkyl) which may be unsubstituted or substituted (e.g., comprising a hydroxy group, an oxo group, or an ionizable nitrogen group as described herein), and/or comprises 1 or 2 heteroatoms selected from N and O. In embodiments, a heteroalkyl comprises an oxo substituent group (e.g., a heteroalkyl comprises
20 an ester group where the oxo is adjacent to an oxygen atom).

[0157] *Heteroalkylene*: The term “heteroalkylene,” as used herein, represents a divalent form of a heteroalkyl group as described herein.

[0158] *Heteroaryl*: The term “heteroaryl,” as used herein, is fully unsaturated heteroatom-containing ring wherein at least one ring atom is a heteroatom such as, but not limited to,
25 nitrogen and oxygen.

[0159] As used herein, “heteroaryl” also refers to a radical of a 5-14 membered monocyclic or polycyclic (e.g., bicyclic or tricyclic) 4n+2 aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4 ring
heteroatoms) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is
30 independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-14 membered heteroaryl”). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl polycyclic ring

systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the

5 heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused polycyclic (aryl/heteroaryl) ring system. Polycyclic heteroaryl groups wherein one ring does not contain a heteroatom (e.g., indolyl, quinolynyl, carbazolyl, and the like) the point of attachment can be on either ring, i.e., either the ring bearing

10 a heteroatom (e.g., 2-indolyl) or the ring that does not contain a heteroatom (e.g., 5-indolyl).

[0160] In some embodiments, a heteroaryl group is a 5-10 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur,

15 nitrogen, boron, silicon, and phosphorus ("5-10 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5-8 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("5-8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5-6

20 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("5-6 membered heteroaryl"). In some embodiments, the 5-6 membered heteroaryl has 1 or more (e.g., 1, 2, or 3) ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some

25 embodiments, the 5-6 membered heteroaryl has 1 or 2 ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heteroaryl has 1 ring heteroatom selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted (an "unsubstituted heteroaryl") or substituted (a "substituted heteroaryl") with one

30 or more substituents. In certain embodiments, the heteroaryl group is an unsubstituted 5-14 membered heteroaryl. In certain embodiments, the heteroaryl group is a substituted 5-14 membered heteroaryl.

- [0161] Exemplary 5-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5-membered heteroaryl groups containing 2 heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5-membered heteroaryl groups containing 3 heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5-membered heteroaryl groups containing 4 heteroatoms include, without limitation, tetrazolyl. Exemplary 6-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyridinyl. Exemplary 6-membered heteroaryl groups containing 2 heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6-membered heteroaryl groups containing 3 or 4 heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing 1 heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6-bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizynyl, and purinyl. Exemplary 6,6-bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl. Exemplary tricyclic heteroaryl groups include, without limitation, phenanthridinyl, dibenzofuranyl, carbazolyl, acridinyl, phenothiazinyl, phenoxazinyl and phenazinyl.
- [0162] As used herein, "heterocyclyl" or "heterocyclic" refers to a radical of a 3- to 14-membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("3-14 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or polycyclic (e.g., a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl") or tricyclic system ("tricyclic heterocyclyl")). and can be saturated or can contain one or more carbon-carbon double or triple bonds. Heterocyclyl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such

instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently unsubstituted (an “unsubstituted heterocyclyl”) or substituted (a “substituted heterocyclyl”) with one or more substituents. In certain embodiments, the heterocyclyl group is an unsubstituted 3-14 membered heterocyclyl. In certain embodiments, the heterocyclyl group is a substituted 3-14 membered heterocyclyl.

[0163] In some embodiments, a heterocyclyl group is a 5-10 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-10 membered heterocyclyl”). In some embodiments, a heterocyclyl group is a 5-8 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-8 membered heterocyclyl”). In some embodiments, a heterocyclyl group is a 5-6 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-6 membered heterocyclyl”). In some embodiments, the 5-6 membered heterocyclyl has 1 or more (e.g., 1, 2, or 3) ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heterocyclyl has 1 or 2 ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heterocyclyl has 1 ring heteroatom selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus.

[0164] Exemplary 3-membered heterocyclyl groups containing 1 heteroatom include, without limitation, aziridinyl, oxiranyl, thiorenlyl. Exemplary 4-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azetidiny, oxetanyl and thietanyl. Exemplary 5-membered heterocyclyl groups containing 1 heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl-2,5-dione. Exemplary 5-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, dioxolanyl, oxathiolanyl and dithiolanyl. Exemplary 5-membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazolinyl, oxadiazolinyl, and thiadiazolinyl. Exemplary 6-membered heterocyclyl groups containing 1 heteroatom include, without limitation, piperidinyl, tetrahydropyranyl, dihydropyridinyl, and thianyl. Exemplary 6-membered heterocyclyl groups containing 2 heteroatoms include, without

limitation, piperazinyl, morpholinyl, dithianyl, dioxanyl. Exemplary 6-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, triazinanyl. Exemplary 7-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8-membered heterocyclyl groups containing 1 heteroatom
5 include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary bicyclic heterocyclyl groups include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, tetrahydrobenzothienyl, tetrahydrobenzofuranyl, tetrahydroindolyl, tetrahydroquinolyl, tetrahydroisoquinolyl, decahydroquinolyl, decahydroisoquinolyl, octahydrochromenyl, octahydroisochromenyl, decahydronaphthyridinyl, decahydro-1,8-
10 naphthyridinyl, octahydropyrrolo[3,2-b]pyrrole, indolinyl, phthalimidyl, naphthalimidyl, chromanyl, chromenyl, 1H-benzo[e][1,4]diazepinyl, 1,4,5,7-tetrahydropyrano[3,4-b] pyrrolyl, 5,6-dihydro-4H-furo[3,2-b]pyrrolyl, 6,7-dihydro-5H-furo[3,2-b]pyranyl, 5,7-dihydro-4H-thieno[2,3-c]pyranyl, 2,3-dihydro-1H-pyrrolo[2,3-b]pyridinyl, 2,3-dihydrofuro[2,3-b]pyridinyl, 4,5,6,7-tetrahydro-1H-pyrrolo-
[2,3-b]pyridinyl, 4,5,6,7-tetrahydrofuro[3,2-c]pyridinyl, 4,5,6,7-tetrahydrothieno [3,2- b]pyridinyl,
15 1,2,3,4-tetrahydro-1,6-naphthyridinyl, and the like.

[0165] *Heterocycloalkyl*: The term “heterocycloalkyl,” as used herein, is a non-aromatic ring wherein at least one atom is a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus, and the remaining atoms are carbon. The heterocycloalkyl group can be substituted or unsubstituted.

20 **[0166]** As understood from the above, alkyl, alkenyl, alkynyl, acyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups, as defined herein, are, in certain embodiments, optionally substituted. Optionally substituted refers to a group which may be substituted or unsubstituted (e.g., “substituted” or “unsubstituted” alkyl, “substituted” or “unsubstituted” alkenyl, “substituted” or “unsubstituted” alkynyl, “substituted” or “unsubstituted” heteroalkyl,
25 “substituted” or “unsubstituted” heteroalkenyl, “substituted” or “unsubstituted” heteroalkynyl, “substituted” or “unsubstituted” carbocyclyl, “substituted” or “unsubstituted” heterocyclyl, “substituted” or “unsubstituted” aryl or “substituted” or “unsubstituted” heteroaryl group. In general, the term “substituted” means that at least one hydrogen present on a group is replaced with a permissible substituent, e.g., a substituent which upon substitution results in a stable
30 compound, e.g., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a “substituted” group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the

same or different at each position. The term "substituted" is contemplated to include substitution with all permissible substituents of organic compounds, any of the substituents described herein that results in the formation of a stable compound. The present invention contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

[0167] Exemplary carbon atom substituents include, but are not limited to, halogen, -CN, -NO₂, -N₃, -SO₂, -SO₃H, -OH, -OR^{aa}, -ON(R^{bb})₂, -N(R^{bb})₂, -N(R^{bb})₃+X⁻, -N(OR^{cc})R^{bb}, -SeH, -SeR^{aa}, -SH, -SR^{aa}, -SSR^{cc}, -C(=O)R^{aa}, -CO₂H, -CHO, -C(OR^{cc})₂, -CO₂R^{aa}, -OC(=O)R^{aa}, -OCO₂R^{aa}, -C(=O)N(R^{bb})₂, -OC(=O)N(R^{bb})₂, -NR^{bb}C(=O)R^{aa}, -NR^{bb}CO₂R^{aa}, -NR^{bb}C(=O)N(R^{bb})₂, -C(=NR^{bb})R^{aa}, -C(=NR^{bb})OR^{aa}, -OC(=NR^{bb})R^{aa}, -OC(=NR^{bb})OR^{aa}, -C(=NR^{bb})N(R^{bb})₂, -OC(=NR^{bb})N(R^{bb})₂, -NR^{bb}C(=NR^{bb})N(R^{bb})₂, -C(=O)NR^{bb}SO₂R^{aa}, -NR^{bb}SO₂R^{aa}, -SO₂N(R^{bb})₂, -SO₂R^{aa}, -SO₂OR^{aa}, -OSO₂R^{aa}, -S(=O)R^{aa}, -OS(=O)R^{aa}, -Si(R^{aa})₃, -OSi(R^{aa})₃, -C(=S)N(R^{bb})₂, -C(=O)SR^{aa}, -C(=S)SR^{aa}, -SC(=S)SR^{aa}, -SC(=O)SR^{aa}, -OC(=O)SR^{aa}, -SC(=O)OR^{aa}, -SC(=O)R^{aa}, -P(=O)₂R^{aa}, -OP(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -OP(=O)(R^{aa})₂, -OP(=O)(OR^{cc})₂, -P(=O)₂N(R^{bb})₂, -OP(=O)₂N(R^{bb})₂, -P(=O)(NR^{bb})₂, -OP(=O)(NR^{bb})₂, -NR^{bb}P(=O)(OR^{cc})₂, -NR^{bb}P(=O)(NR^{bb})₂, -P(R^{cc})₂, -P(R^{cc})₃, -OP(R^{cc})₂, -OP(R^{cc})₃, -B(R^{aa})₂, -B(OR^{cc})₂, -BR^{aa}(OR^{cc}), C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₄ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, =NN(R^{bb})₂, =NNR^{bb}C(=O)R^{aa}, =NNR^{bb}C(=O)OR^{aa}, =NNR^{bb}S(=O)₂R^{aa}, =NR^{bb}, or =NOR^{cc};

[0168] each instance of R^{aa} is, independently, selected from C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{aa} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

[0169] each instance of R^{bb} is, independently, selected from hydrogen, -OH, -OR^{aa}, -N(R^{cc})₂, -CN, -C(=O)R^{aa}, -C(=O)N(R^{cc})₂, -CO₂R^{aa}, -SO₂R^{aa}, -C(=NR^{cc})OR^{aa}, -C(=NR^{cc})N(R^{cc})₂, -SO₂N(R^{cc})₂, -SO₂R^{cc}, -SO₂OR^{cc}, -SOR^{aa}, -C(=S)N(R^{cc})₂, -C(=O)SR^{cc}, -C(=S)SR^{cc}, -P(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -P(=O)₂N(R^{cc})₂, -P(=O)(NR^{cc})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered

heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{bb} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

- 5 **[0170]** each instance of R^{cc} is, independently, selected from hydrogen, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{cc} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

- 10 **[0171]** each instance of R^{dd} is, independently, selected from halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OR^{ee}, -ON(R^{ff})₂, -N(R^{ff})₂, -N(R^{ff})₃+X⁻, -N(OR^{ee})R^{ff}, -SH, -SR^{ee}, -SSR^{ee}, -C(=O)R^{ee}, -CO₂H, -CO₂R^{ee}, -OC(=O)R^{ee}, -OCO₂R^{ee}, -C(=O)N(R^{ff})₂, -OC(=O)N(R^{ff})₂, -NR^{ff}C(=O)R^{ee}, -NR^{ff}CO₂R^{ee}, -NR^{ff}C(=O)N(R^{ff})₂, -C(=NR^{ff})OR^{ee}, -OC(=NR^{ff})R^{ee}, -OC(=NR^{ff})OR^{ee}, -C(=NR^{ff})N(R^{ff})₂, -OC(=NR^{ff})N(R^{ff})₂, -NR^{ff}C(=NR^{ff})N(R^{ff})₂, -NR^{ff}SO₂R^{ee}, -SO₂N(R^{ff})₂, -SO₂R^{ee}, -SO₂OR^{ee}, -OSO₂R^{ee}, -S(=O)R^{ee}, -Si(R^{ee})₃, -OSi(R^{ee})₃, -C(=S)N(R^{ff})₂, -C(=O)SR^{ee}, -C(=S)SR^{ee}, -SC(=S)SR^{ee}, -P(=O)₂R^{ee}, -P(=O)(R^{ee})₂, -OP(=O)(R^{ee})₂, -OP(=O)(OR^{ee})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-10 membered heterocyclyl, C₆-C₁₀ aryl, 5-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups, or two geminal R^{dd} substituents can be joined to form =O or =S;

[0172] each instance of R^{ee} is, independently, selected from C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, C₆-C₁₀ aryl, 3-10 membered heterocyclyl, and 3-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups;

- 25 **[0173]** each instance of R^{ff} is, independently, selected from hydrogen, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-10 membered heterocyclyl, C₆-C₁₀ aryl and 5-10 membered heteroaryl, or two R^{ff} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups; and

[0174] each instance of R^{gg} is, independently, halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OC₁-C₅₀ alkyl, -ON(C₁-C₅₀ alkyl)₂, -N(C₁-C₅₀ alkyl)₂, -N(C₁-C₅₀ alkyl)₃+X⁻, -NH(C₁-C₅₀ alkyl)₂+X⁻, -NH₂(C₁-

- C₅₀ alkyl) +X⁻, -NH₃⁺X⁻, -N(OC₁-C₅₀ alkyl)(C₁-C₅₀ alkyl), -N(OH)(C₁-C₅₀ alkyl), -NH(OH), -SH, -SC₁-C₅₀ alkyl, -SS(C₁-C₅₀ alkyl), -C(=O)(C₁-C₅₀ alkyl), -CO₂H, -CO₂(C₁-C₅₀ alkyl), -OC(=O)(C₁-C₅₀ alkyl), -OCO₂(C₁-C₅₀ alkyl), -C(=O)NH₂, -C(=O)N(C₁-C₅₀ alkyl)₂, -OC(=O)NH(C₁-C₅₀ alkyl), -NHC(=O)(C₁-C₅₀ alkyl), -N(C₁-C₅₀ alkyl)C(=O)(C₁-C₅₀ alkyl), -NHCO₂(C₁-C₅₀ alkyl), -NHC(=O)N(C₁-C₅₀ alkyl)₂, -
- 5 NHC(=O)NH(C₁-C₅₀ alkyl), -NHC(=O)NH₂, -C(=NH)O(C₁-C₅₀ alkyl), -OC(=NH)(C₁-C₅₀ alkyl), -OC(=NH)OC₁-C₅₀ alkyl, -C(=NH)N(C₁-C₅₀ alkyl)₂, -C(=NH)NH(C₁-C₅₀ alkyl), -C(=NH)NH₂, -OC(=NH)N(C₁-C₅₀ alkyl)₂, -OC(NH)NH(C₁-C₅₀ alkyl), -OC(NH)NH₂, -NHC(NH)N(C₁-C₅₀ alkyl)₂, -NHC(=NH)NH₂, -NHOSO₂(C₁-C₅₀ alkyl), -SO₂N(C₁-C₅₀ alkyl)₂, -SO₂NH(C₁-C₅₀ alkyl), -SO₂NH₂, -SO₂(C₁-C₅₀ alkyl), -SO₂O(C₁-C₅₀ alkyl), -OSO₂(C₁-C₆ alkyl), -SO(C₁-C₆ alkyl), -Si(C₁-C₅₀ alkyl)₃, -OSi(C₁-C₆ alkyl)₃, -
- 10 C(=S)N(C₁-C₅₀ alkyl)₂, C(=S)NH(C₁-C₅₀ alkyl), C(=S)NH₂, -C(=O)S(C₁-C₆ alkyl), -C(=S)S(C₁-C₆ alkyl), -SC(=S)S(C₁-C₆ alkyl), -P(=O)₂(C₁-C₅₀ alkyl), -P(=O)(C₁-C₅₀ alkyl)₂, -OP(=O)(C₁-C₅₀ alkyl)₂, -OP(=O)(OC₁-C₅₀ alkyl)₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, C₆-C₁₀ aryl, 3-10 membered heterocyclyl, 5-10 membered heteroaryl; or two geminal R^{BB} substituents can be joined to form =O or =S; wherein X⁻ is a counterion.
- 15 **[0175]** As used herein, the term “halo” or “halogen” refers to fluorine (fluoro, -F), chlorine (chloro, -Cl), bromine (bromo, -Br), or iodine (iodo, -I).
- [0176]** As used herein, a “counterion” is a negatively charged group associated with a positively charged quarternary amine in order to maintain electronic neutrality. Exemplary counterions include halide ions (e.g., F⁻, Cl⁻, Br⁻, I⁻), NO₃⁻, ClO₄⁻, OH⁻, H₂PO₄⁻, HSO₄⁻, sulfonate ions (e.g.,
- 20 methanesulfonate, trifluoromethanesulfonate, p-toluenesulfonate, benzenesulfonate, 10-camphor sulfonate, naphthalene-2-sulfonate, naphthalene-1-sulfonic acid-5-sulfonate, ethan-1-sulfonic acid-2-sulfonate, and the like), and carboxylate ions (e.g., acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).
- [0177]** Nitrogen atoms can be substituted or unsubstituted as valency permits, and include
- 25 primary, secondary, tertiary, and quarternary nitrogen atoms. Exemplary nitrogen atom substituents include, but are not limited to, hydrogen, -OH, -OR^{aa}, -N(R^{cc})₂, -CN, -C(=O)R^{aa}, -C(=O)N(R^{cc})₂, -CO₂R^{aa}, -SO₂R^{aa}, -C(=NR^{bb})R^{aa}, -C(=NR^{cc})OR^{aa}, -C(=NR^{cc})N(R^{cc})₂, -SO₂N(R^{cc})₂, -SO₂R^{cc}, -SO₂OR^{cc}, -SOR^{aa}, -C(=S)N(R^{cc})₂, -C(=O)SR^{cc}, -C(=S)SR^{cc}, -P(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -P(=O)₂N(R^{cc})₂, -P(=O)(NR^{cc})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered
- 30 heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{cc} groups, together with the N atom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl

is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc} and R^{dd} are as defined above.

[0178] In certain embodiments, the substituent present on a nitrogen atom is a nitrogen protecting group (also referred to as an amino protecting group). Nitrogen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[0179] For example, nitrogen protecting groups such as amide groups (e.g., -C(=O)R^{aa}) include, but are not limited to, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, N-benzoylphenylalanyl derivative, benzamide, p-phenylbenzamide, o-nitrophenylacetamide, o-nitrophenoxyacetamide, acetoacetamide, (N'-dithiobenzoyloxyacylamino)acetamide, 3-(p-hydroxyphenyl)propanamide, 3-(o-nitrophenyl)propanamide, 2-methyl-2-(o-nitrophenoxy)propanamide, 2-methyl-2-(o-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, o-nitrocinnamide, N-acetylmethionine derivative, o-nitrobenzamide and o-(benzoyloxymethyl)benzamide.

[0180] Nitrogen protecting groups such as carbamate groups (e.g., -C(=O)OR^{aa}) include, but are not limited to, methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-t-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilyl ethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamanty1)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-t-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenyl)ethyl carbamate (Bpoc), 1-(3,5-di-t-butylphenyl)-1-methylethyl carbamate (t-Bumeoc), 2-(2'-and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(N,N-dicyclohexylcarboxamido)ethyl carbamate, t-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, N-hydroxypiperidinyl carbamate, alkyl dithio carbamate, benzyl carbamate (Cbz), p-methoxybenzyl carbamate (Moz), p-nitobenzyl carbamate, p-bromobenzyl carbamate, p-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (MsZ), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-

- methysulfonyl ethyl carbamate, 2-(p-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, m-chloro-p-acyloxybenzyl carbamate, p-
- 5 (dihydroxyboryl)benzyl carbamate, 5-benzisoxazolymethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), m-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, o-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(o-nitrophenyl)methyl carbamate, t-amyl carbamate, S-benzyl thiocarbamate, p-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p-
- 10 decyloxybenzyl carbamate, 2,2-dimethoxyacetylvinyl carbamate, o-(N,N-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(N,N-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p-(p'-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-
- 15 methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(p-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, p-(phenylazo)benzyl carbamate, 2,4,6-tri-t-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, and 2,4,6-trimethylbenzyl carbamate.
- 20 **[0181]** Nitrogen protecting groups such as sulfonamide groups (e.g., -S(=O)₂R^{aa}) include, but are not limited to, p-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-
- 25 4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β-trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.
- [0182]** Other nitrogen protecting groups include, but are not limited to, phenothiazinyl-(10)-
- 30 acyl derivative, N'-p-toluenesulfonylaminoacyl derivative, N'-phenylaminothioacyl derivative, N-benzoylphenylalanyl derivative, N-acetylmethionine derivative, 4,5-diphenyl-3-oxazolin-2-one, N-phthalimide, N-dithiasuccinimide (Dts), N-2,3-diphenylmaleimide, N-2,5-dimethylpyrrole, N-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-

- triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, N-methylamine, N-allylamine, N-[2-(trimethylsilyl)ethoxy]methylamine (SEM), N-3-acetoxypyrrolamine, N-(1-isopropyl-4-nitro-2-oxo-3-pyrroline-3-yl)amine, quaternary ammonium salts, N-benzylamine, N-di(4-methoxyphenyl)methylamine, N-5-dibenzosuberylamine, 5 N-triphenylmethylamine (Tr), N-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), N-9-phenylfluorenylamine (PhF), N-2,7-dichloro-9-fluorenylmethyleneamine, N-ferrocenylmethylamino (Fcm), N-2-picolylamino N'-oxide, N-1,1-dimethylthiomethyleneamine, N-benzylideneamine, N-p-methoxybenzylideneamine, N-diphenylmethyleneamine, N-[(2-pyridyl)mesityl]methyleneamine, N-(N',N'-dimethylaminomethylene)amine, N,N'-10 isopropylidenediamine, N-p-nitrobenzylideneamine, N-salicylideneamine, N-5-chlorosalicylideneamine, N-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, N-cyclohexylideneamine, N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, N-borane derivative, N-diphenylborinic acid derivative, N-[phenyl(pentaacylchromium- or tungsten)acyl]amine, N-copper chelate, N-zinc chelate, N-nitroamine, N-nitrosoamine, amine N-oxide, diphenylphosphinamide 15 (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, and 3-nitropyridinesulfenamide (Npys).
- 20 **[0183]** In certain embodiments, the substituent present on an oxygen atom is an oxygen protecting group (also referred to as a hydroxyl protecting group). Oxygen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.
- 25 **[0184]** Exemplary oxygen protecting groups include, but are not limited to, methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 30 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-

- y1 (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl (Bn), p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberil, triphenylmethyl, α -naphthylidiphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodisulfuran-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyl dimethylsilyl (TBDMS), t-butyl diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butyl methoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl)ethyl carbonate (Psec), 2-(triphenylphosphonio)ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenate, o-(methoxyacyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-

tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzyisulfonate, and tosylate (Ts).

5 [0185] In certain embodiments, the substituent present on a sulfur atom is a sulfur protecting group (also referred to as a thiol protecting group). Sulfur protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[0186] Exemplary sulfur protecting groups include, but are not limited to, alkyl, benzyl, p-methoxybenzyl, 2,4,6-trimethylbenzyl, 2,4,6-trimethoxybenzyl, o-hydroxybenzyl, p-hydroxybenzyl, o-acetoxybenzyl, p-acetoxybenzyl, p-nitrobenzyl, 4-picolyl, 2-quinolinylmethyl, 2-picolyl N-oxido, 9-anthrylmethyl, 9-fluorenylmethyl, xanthenyl, ferrocenylmethyl, diphenylmethyl, bis(4-methoxyphenyl)methyl, 5-dibenzosuberyl, triphenylmethyl, diphenyl-4-pyridylmethyl, phenyl, 2,4-dinitrophenyl, t-butyl, 1-adamantyl, methoxymethyl (MOM), isobutoxymethyl, benzyloxymethyl, 15 2-tetrahydropyranyl, benzylthiomethyl, phenylthiomethyl, thiazolidino, acetamidomethyl, trimethylacetamidomethyl, benzamidomethyl, allyloxycarbonylaminomethyl, phenylacetamidomethyl, phthalimidomethyl, acetylmethyl, carboxymethyl, cyanomethyl, (2-nitro-1-phenyl)ethyl, 2-(2,4-dinitrophenyl)ethyl, 2-cyanoethyl, 2-(Trimethylsilyl)ethyl, 2,2-bis(carboethoxy)ethyl, (1-m-nitrophenyl-2-benzoyl)ethyl, 2-phenylsulfonyl, 2-(4-methylphenylsulfonyl)-2-methylprop-2-yl, acetyl, benzoyl, trifluoroacetyl, N-[[[(p-biphenyl)isopropoxy]carbonyl]-N-methyl]-γ-aminothiobutyrate, 2,2,2-trichloroethoxycarbonyl, 20 t-butoxycarbonyl, benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, N-ethyl, N-methoxymethyl, sulfonate, sulfenylthiocarbonate, 3-nitro-2-pyridinesulfonyl sulfide, oxathiolone.

25 **Compounds of the Invention**

[0187] Liposomal-based vehicles are considered an attractive carrier for therapeutic agents and remain subject to continued development efforts. While liposomal-based vehicles that comprise certain lipid components have shown promising results with regard to encapsulation, stability and site localization, there remains a great need for improvement of liposomal-based delivery 30 systems. For example, a significant drawback of liposomal delivery systems relates to the construction of liposomes that have sufficient cell culture or in vivo stability to reach desired

target cells and/or intracellular compartments, and the ability of such liposomal delivery systems to efficiently release their encapsulated materials to such target cells.

[0188] Efficient delivery of liposome-encapsulated nucleic acids therefore remains an active area of research. Cationic lipid components play an important role in facilitating effective encapsulation of nucleic acids during the loading of liposomes. In addition, cationic lipids may play an important role in the efficient release of the nucleic acid cargo from the liposome into the cytoplasm of a target cell. Various cationic lipids suitable for *in vivo* use have been discovered. However, there remains a need to identify lipids that can be synthesized efficiently and cheaply without the formation of potentially toxic by-products.

[0189] In particular, there remains a need for improved lipid compounds that demonstrate improved pharmacokinetic properties and which are capable of delivering macromolecules, such as nucleic acids, to a wide variety cell types and tissues with enhanced efficiency. Importantly, there also remains a particular need for novel lipid compounds that are characterized as having reduced toxicity and are capable of efficiently delivering encapsulated nucleic acids and polynucleotides to targeted cells, tissues and organs.

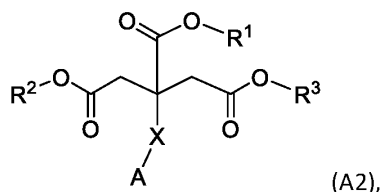
[0190] Described herein a novel class of tricine and citric acid-based cationic lipid compounds for improved in vivo delivery of therapeutic agents, such as nucleic acids. In particular, a tricine and citric acid-based cationic lipid described herein may be used as a cationic lipid, optionally with other lipids, to formulate a lipid-based nanoparticle (*e.g.*, liposome) for encapsulating therapeutic agents, such as nucleic acids (*e.g.*, DNA, siRNA, mRNA, microRNA) for therapeutic use.

[0191] In embodiments, compounds of the invention as described herein can provide one or more desired characteristics or properties. That is, in certain embodiments, compounds of the invention as described herein can be characterized as having one or more properties that afford such compounds advantages relative to other similarly classified lipids. For example, compounds disclosed herein can allow for the control and tailoring of the properties of liposomal compositions (*e.g.*, lipid nanoparticles) of which they are a component. In particular, compounds disclosed herein can be characterized by enhanced transfection efficiencies and their ability to provoke specific biological outcomes. Such outcomes can include, for example enhanced cellular uptake, endosomal/lysosomal disruption capabilities and/or promoting the release of encapsulated materials (*e.g.*, polynucleotides) intracellularly. Additionally, the compounds disclosed herein have advantageous pharmacokinetic properties, biodistribution, and efficiency (*e.g.*, due to the different disassociate rates of the polymer group used).

[0192] Exemplary compounds include those described herein. Variables described for a formula may be according to any permitted structure or value as recited for any other formula or embodiment described herein, and any combinations thereof.

Formula (A2)

- 5 [0193] In one aspect, the invention features a cationic lipid having a structure according to Formula (A2):

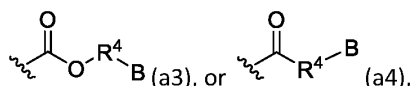
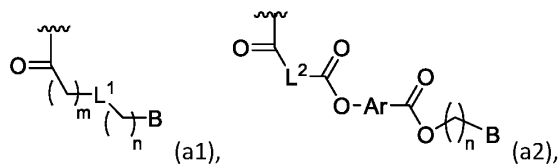


or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

- 10 each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, C_4 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged

- 15 nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L^1 is a carbonyl, ester, or amide;

L^2 is C_2 - C_{10} alkylene or a C_2 - C_{10} alkenylene;

- 20 Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 ;

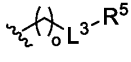
R^4 is C_1 - C_{10} alkylene;

and wherein A is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently a C_4 -

C_{30} heteroalkyl comprising a disulfide bond or having a structure that is ,

wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

[0194] In embodiments, **A** is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently

a C_4 - C_{30} heteroalkyl comprising a disulfide bond or having a structure that is , wherein
 5 L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

[0195] In embodiments, **X** is independently O.

[0196] In embodiments, **X** is independently NH.

[0197] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl. In embodiments,
 10 each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkyl.

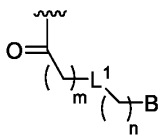
[0198] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkenyl.

15 [0199] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkynyl.

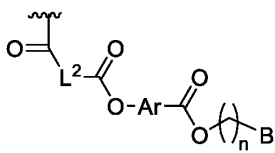
[0200] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30}
 20 heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

[0201] In embodiments, **B** is independently an ionizable nitrogen-containing group.

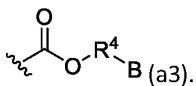
[0202] In embodiments, **B** is independently a permanently charged nitrogen group.



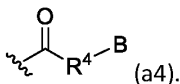
[0203] In embodiments, **A** is (a1).



[0204] In embodiments, **A** is



[0205] In embodiments, **A** is



[0206] In embodiments, **A** is

[0207] In embodiments, **m** is an integer of 2-10.

5 [0208] In embodiments, **n** is an integer of 2-10.

[0209] In embodiments, **L**¹ is a carbonyl.

[0210] In embodiments, **L**¹ is an ester. In embodiments, **L**¹ is $-\text{OC}(\text{O})-$. In embodiments, **L**¹ is $-(\text{O})\text{CO}-$.

10 [0211] In embodiments, **L**¹ is an amide. In embodiments, **L**¹ is $-\text{NHC}(\text{O})-$. In embodiments, **L**¹ is $-\text{C}(\text{O})\text{NH}-$.

[0212] In embodiments, **L**² is C_2 - C_{10} alkylene. In embodiments, **L**² is unsubstituted C_2 - C_{10} alkylene. In embodiments, **L**² is substituted C_2 - C_{10} alkylene.

[0213] In embodiments, **L**² is C_2 - C_{10} alkenylene. In embodiments, **L**² is unsubstituted C_2 - C_{10} alkenylene. In embodiments, **L**² is substituted C_2 - C_{10} alkenylene.

15 [0214] In embodiments, **Ar** is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is unsubstituted phenylene. In embodiments, **Ar** is phenylene comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-3 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-2 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising one substituent selected from halogen, OCH_3 , and CH_3 .

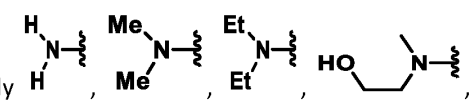
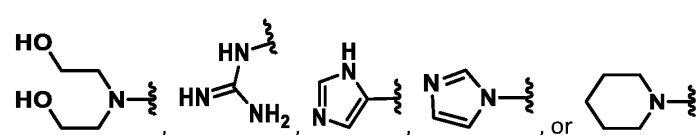
[0215] In embodiments, **R**⁴ is unsubstituted C_1 - C_{10} alkylene. In embodiments, **R**⁴ is substituted C_1 - C_{10} alkylene.

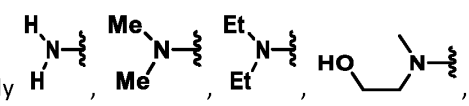
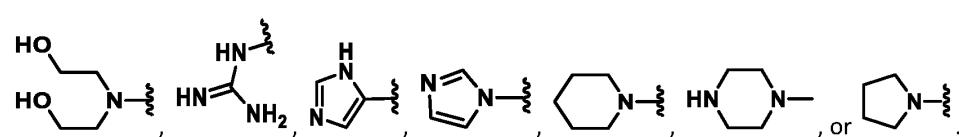
25 [0216] In embodiments, **X** is O and/or **B** is independently an ionizable nitrogen-containing group. In embodiments, **X** is O and **B** is independently an ionizable nitrogen-containing group.

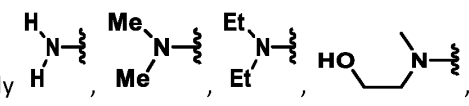
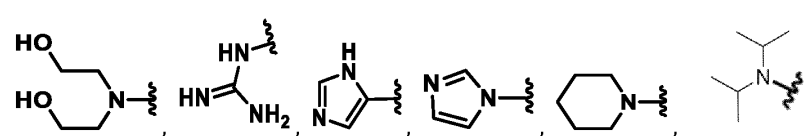
[0217] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, or C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is unsubstituted. In embodiments, each of R^1 , R^2 , and R^3 is substituted (e.g., comprising 1-3 substituents as described herein).

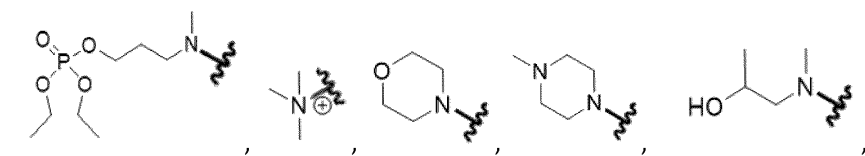
[0218] In embodiments, each of R^1 , R^2 , and R^3 is independently a C_4 - C_{30} heteroalkyl comprising

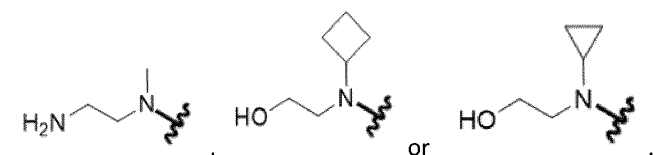
- 5 a disulfide bond or having a structure that is $\text{---}(\text{---})_{\text{o}}\text{---}L^3\text{---}R^5$, wherein L^3 is OC(O) , CO_2 , or (O)CO ; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is OC(O) , CO_2 , or (O)CO ; o is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

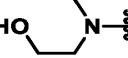
[0219] In embodiments, **B** is independently , .

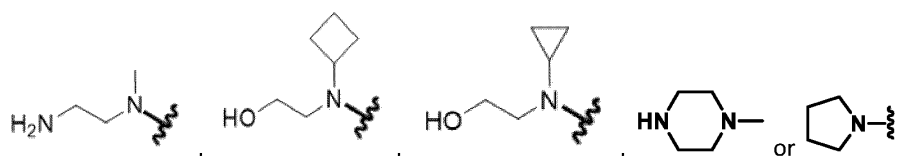
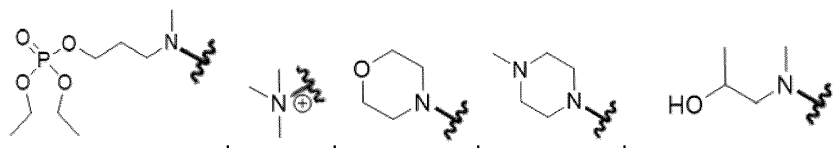
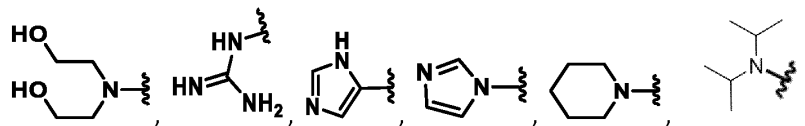
10 [0220] In embodiments, **B** is independently , .

[0221] In embodiments, **B** is independently , .



15 

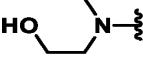
[0222] In embodiments, **B** is independently , , , ,

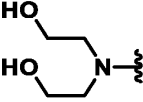


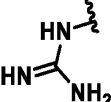
5 [0223] In embodiments, **B** is independently .

[0224] In embodiments, **B** is independently .

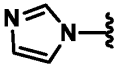
[0225] In embodiments, **B** is independently .

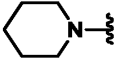
[0226] In embodiments, **B** is independently .

[0227] In embodiments, **B** is independently .

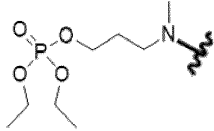
10 [0228] In embodiments, **B** is independently .

[0229] In embodiments, **B** is independently .

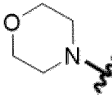
[0230] In embodiments, **B** is independently  .

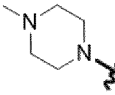
[0231] In embodiments, **B** is independently  .

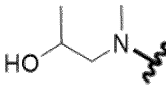
[0232] In embodiments, **B** is independently  .

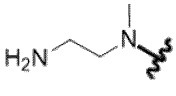
[0233] In embodiments, **B** is independently  .

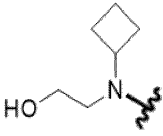
5 [0234] In embodiments, **B** is independently  .

[0235] In embodiments, **B** is independently  .

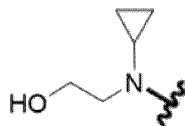
[0236] In embodiments, **B** is independently  .

[0237] In embodiments, **B** is independently  .

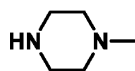
[0238] In embodiments, **B** is independently  .

10 [0239] In embodiments, **B** is independently  .

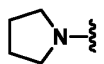
[0240] In embodiments, **B** is independently



[0241] In embodiments, **B** is independently



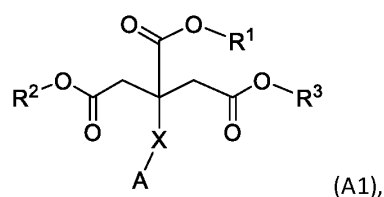
[0242] In embodiments, **B** is independently



5

Formula (A1)

[0243] In one aspect, the invention features a cationic lipid having a structure according to Formula (A1):



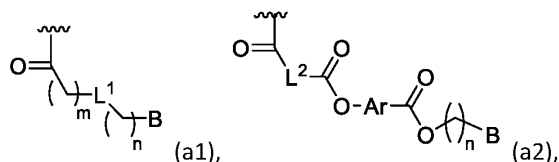
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or a pharmaceutically acceptable salt thereof, wherein

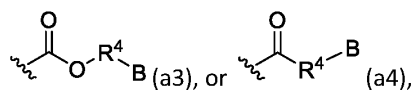
X is independently O or NH;

each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

A is a substructure selected from:



15



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

20

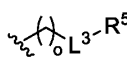
L¹ is a carbonyl, ester, or amide;

L^2 is C_2 - C_{10} alkylene or a C_2 - C_{10} alkenylene;

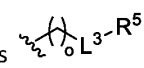
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 ;

R^4 is C_1 - C_{10} alkylene;

5 and wherein **A** is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently a C_4 -

C_{30} heteroalkyl comprising a disulfide bond or having a structure that is , wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

[0244] In embodiments, **A** is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently

10 a C_4 - C_{30} heteroalkyl comprising a disulfide bond or having a structure that is , wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

[0245] In embodiments, **X** is independently O.

[0246] In embodiments, **X** is independently NH.

15 [0247] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkyl.

[0248] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkenyl. In embodiments, each of R^1 ,

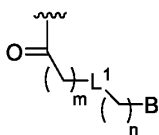
20 R^2 , and R^3 is independently substituted C_4 - C_{30} alkenyl.

[0249] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkynyl.

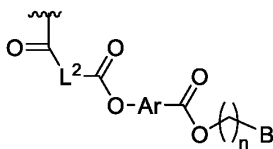
[0250] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

[0251] In embodiments, **B** is independently an ionizable nitrogen-containing group.

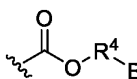
[0252] In embodiments, **B** is independently a permanently charged nitrogen group.



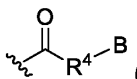
[0253] In embodiments, **A** is (a1).



[0254] In embodiments, **A** is (a2).



[0255] In embodiments, **A** is (a3).



[0256] In embodiments, **A** is (a4).

5 [0257] In embodiments, **m** is an integer of 2-10.

[0258] In embodiments, **n** is an integer of 2-10.

[0259] In embodiments, **L¹** is a carbonyl.

[0260] In embodiments, **L¹** is an ester. In embodiments, **L¹** is $-\text{OC}(\text{O})-$. In embodiments, **L¹** is $-(\text{O})\text{CO}-$.

10 [0261] In embodiments, **L¹** is an amide. In embodiments, **L¹** is $-\text{NHC}(\text{O})-$. In embodiments, **L¹** is $-\text{C}(\text{O})\text{NH}-$.

[0262] In embodiments, **L²** is $\text{C}_2\text{-C}_{10}$ alkylene. In embodiments, **L²** is unsubstituted $\text{C}_2\text{-C}_{10}$ alkylene. In embodiments, **L²** is substituted $\text{C}_2\text{-C}_{10}$ alkylene.

15 [0263] In embodiments, **L²** is $\text{C}_2\text{-C}_{10}$ alkenylene. In embodiments, **L²** is unsubstituted $\text{C}_2\text{-C}_{10}$ alkenylene. In embodiments, **L²** is substituted $\text{C}_2\text{-C}_{10}$ alkenylene.

[0264] In embodiments, **Ar** is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is unsubstituted phenylene. In embodiments, **Ar** is phenylene comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-3 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-2 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising one substituent selected from halogen, OCH_3 , and CH_3 .

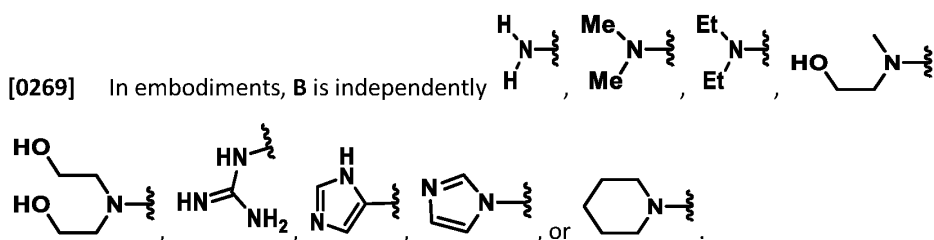
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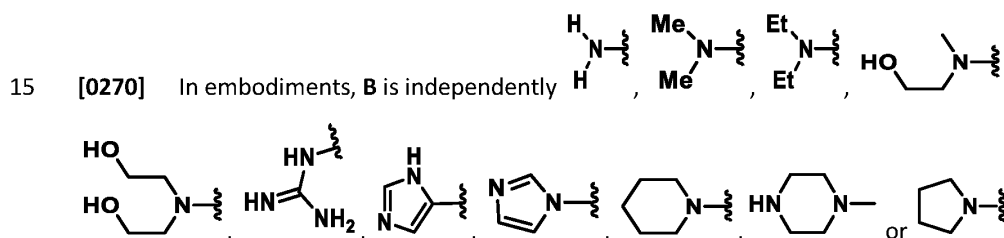
[0265] In embodiments, R^4 is unsubstituted C_1 - C_{10} alkylene. In embodiments, R^4 is substituted C_1 - C_{10} alkylene.

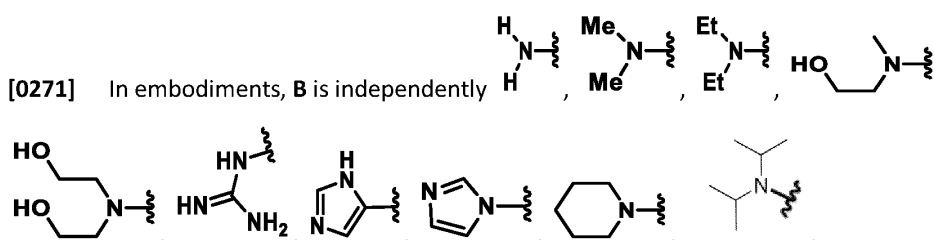
[0266] In embodiments, X is O and/or B is independently an ionizable nitrogen-containing group. In embodiments, X is O and B is independently an ionizable nitrogen-containing group.

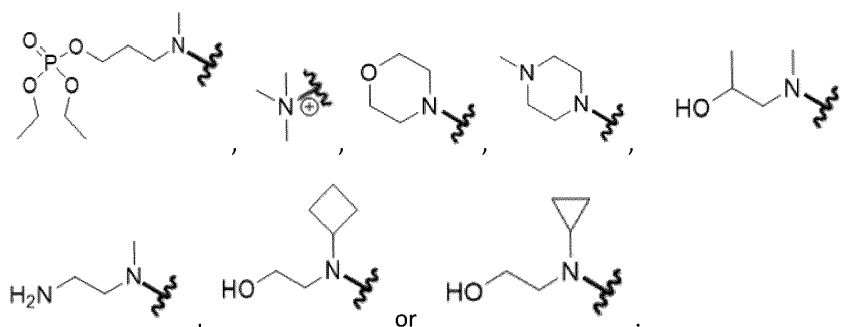
[0267] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, or C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is unsubstituted. In embodiments, each of R^1 , R^2 , and R^3 is substituted (e.g., comprising 1-3 substituents as described herein).

[0268] In embodiments, each of R^1 , R^2 , and R^3 is independently a C_4 - C_{30} heteroalkyl comprising a disulfide bond or having a structure that is $\text{---}(\text{---})_{\text{o}}\text{---}L^3\text{---}R^5$, wherein L^3 is OC(O) , CO_2 , or (O)CO ; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is OC(O) , CO_2 , or (O)CO ; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

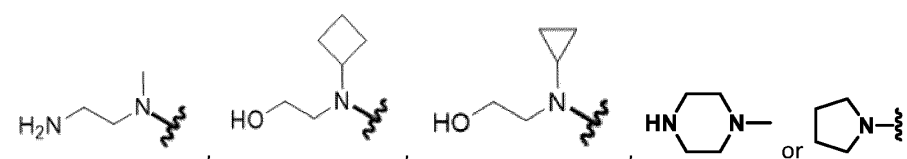
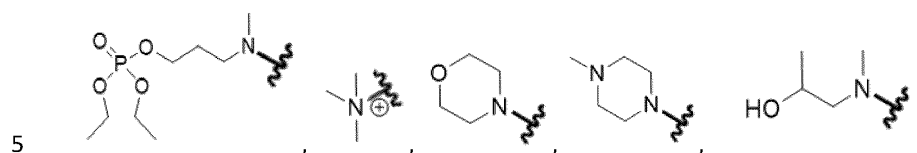
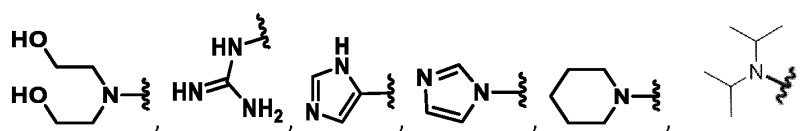
[0269] In embodiments, B is independently 

[0270] In embodiments, B is independently 

[0271] In embodiments, B is independently 



[0272] In embodiments, **B** is independently , , , .



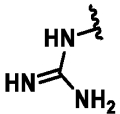
[0273] In embodiments, **B** is independently .

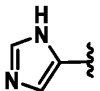
[0274] In embodiments, **B** is independently .

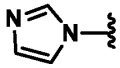
[0275] In embodiments, **B** is independently .

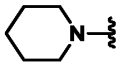
10 [0276] In embodiments, **B** is independently .

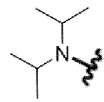
[0277] In embodiments, **B** is independently .

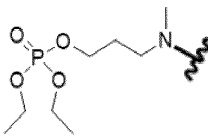
[0278] In embodiments, **B** is independently 

[0279] In embodiments, **B** is independently 

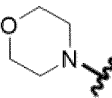
[0280] In embodiments, **B** is independently 

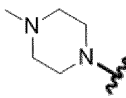
[0281] In embodiments, **B** is independently 

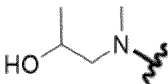
5 [0282] In embodiments, **B** is independently 

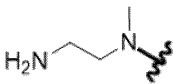
[0283] In embodiments, **B** is independently 

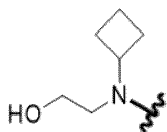
[0284] In embodiments, **B** is independently 

[0285] In embodiments, **B** is independently 

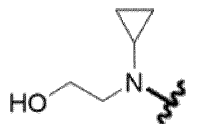
[0286] In embodiments, **B** is independently 

10 [0287] In embodiments, **B** is independently 

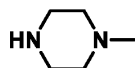
[0288] In embodiments, **B** is independently 



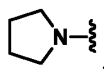
[0289] In embodiments, **B** is independently



[0290] In embodiments, **B** is independently



[0291] In embodiments, **B** is independently

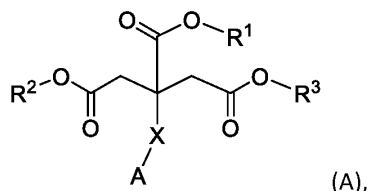


[0292] In embodiments, **B** is independently

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Formula (A)

[0293] In one aspect, the invention features a cationic lipid having a structure according to Formula (A):



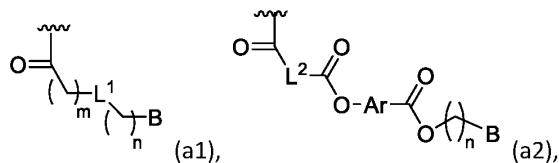
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(A),

or a pharmaceutically acceptable salt thereof, wherein

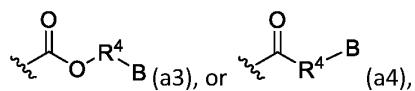
X is independently O or NH;

each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;



15

A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

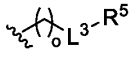
5 **L**¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH₃, and CH₃; and

R⁴ is C₂-C₁₀ alkylene.

10 **[0294]** In embodiments, **A** is only substructure (a4) when each of **R**¹, **R**², and **R**³ is independently

a C₄-C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is , wherein **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R**⁵ is C₄-C₂₄ alkyl; or **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R**⁵ is C₁-C₅ alkyl.

15 **[0295]** In embodiments, **X** is independently O.

[0296] In embodiments, **X** is independently NH.

[0297] In embodiments, each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl. In embodiments, each of **R**¹, **R**², and **R**³ is independently unsubstituted C₄-C₃₀ alkyl. In embodiments, each of **R**¹, **R**², and **R**³ is independently substituted C₄-C₃₀ alkyl.

20 **[0298]** In embodiments, each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkenyl. In embodiments, each of **R**¹, **R**², and **R**³ is independently unsubstituted C₄-C₃₀ alkenyl. In embodiments, each of **R**¹, **R**², and **R**³ is independently substituted C₄-C₃₀ alkenyl.

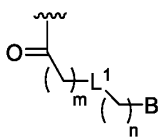
[0299] In embodiments, each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkynyl. In embodiments, each of **R**¹, **R**², and **R**³ is independently unsubstituted C₄-C₃₀ alkynyl. In embodiments, each of **R**¹,

25 **R**², and **R**³ is independently substituted C₄-C₃₀ alkynyl.

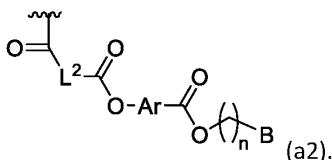
[0300] In embodiments, each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ heteroalkyl (e.g., C₆-C₃₀ heteroalkyl). In embodiments, each of **R**¹, **R**², and **R**³ is independently unsubstituted C₄-C₃₀ heteroalkyl (e.g., unsubstituted C₆-C₃₀ heteroalkyl). In embodiments, each of **R**¹, **R**², and **R**³ is independently substituted C₄-C₃₀ heteroalkyl (e.g., substituted C₆-C₃₀ heteroalkyl).

30 **[0301]** In embodiments, **B** is independently an ionizable nitrogen-containing group.

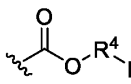
[0302] In embodiments, **B** is independently a permanently charged nitrogen group.

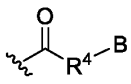


[0303] In embodiments, **A** is (a1).



[0304] In embodiments, **A** is (a2).

[0305] In embodiments, **A** is  (a3).

5 [0306] In embodiments, **A** is  (a4).

[0307] In embodiments, **m** is an integer of 2-10.

[0308] In embodiments, **n** is an integer of 2-10.

[0309] In embodiments, **L¹** is a carbonyl.

10 [0310] In embodiments, **L¹** is an ester. In embodiments, **L¹** is --OC(O)-- . In embodiments, **L¹** is --(O)CO-- .

[0311] In embodiments, **L¹** is an amide. In embodiments, **L¹** is --NHC(O)-- . In embodiments, **L¹** is --C(O)NH-- .

[0312] In embodiments, **L²** is $\text{C}_2\text{--C}_{10}$ alkylene. In embodiments, **L²** is unsubstituted $\text{C}_2\text{--C}_{10}$ alkylene. In embodiments, **L²** is substituted $\text{C}_2\text{--C}_{10}$ alkylene.

15 [0313] In embodiments, **L²** is $\text{C}_2\text{--C}_{10}$ alkenylene. In embodiments, **L²** is unsubstituted $\text{C}_2\text{--C}_{10}$ alkenylene. In embodiments, **L²** is substituted $\text{C}_2\text{--C}_{10}$ alkenylene.

[0314] In embodiments, **Ar** is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is unsubstituted phenylene. In embodiments, **Ar** is phenylene comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-3 substituents independently selected from halogen, OCH_3 , and CH_3 . In embodiments, **Ar** is phenylene comprising 1-2

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substituents independently selected from halogen, OCH₃, and CH₃. In embodiments, **Ar** is phenylene comprising one substituent selected from halogen, OCH₃, and CH₃.

[0315] In embodiments, **R⁴** is unsubstituted C₂-C₁₀ alkylene. In embodiments, **R⁴** is substituted C₂-C₁₀ alkylene.

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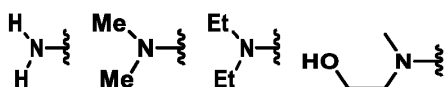
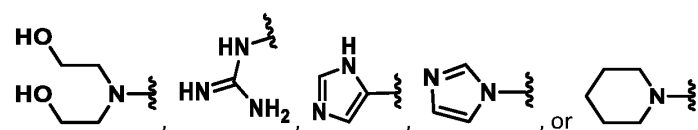
[0316] In embodiments, **X** is O and/or **B** is independently an ionizable nitrogen-containing group. In embodiments, **X** is O and **B** is independently an ionizable nitrogen-containing group.

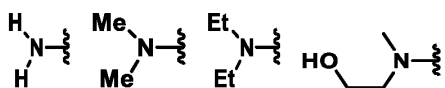
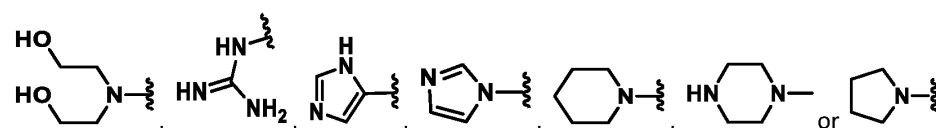
[0317] In embodiments, each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, or C₄-C₃₀ alkynyl. In embodiments, each of **R¹**, **R²**, and **R³** is unsubstituted. In embodiments, each of **R¹**, **R²**, and **R³** is substituted (e.g., comprising 1-3 substituents as described herein).

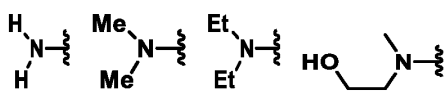
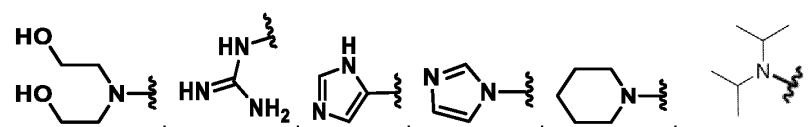
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[0318] In embodiments, each of **R¹**, **R²**, and **R³** is independently a C₄-C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is $\text{---}(\text{---})_{\text{o}}\text{---L}^3\text{---R}^5$, wherein **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl; or **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₅ alkyl.

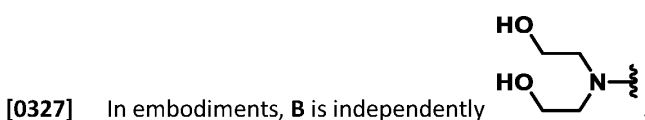
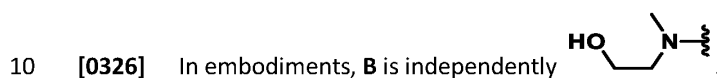
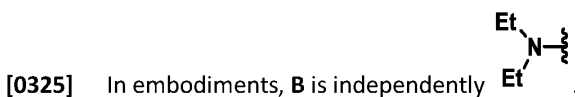
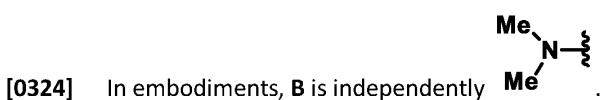
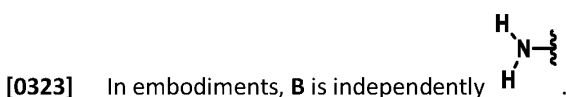
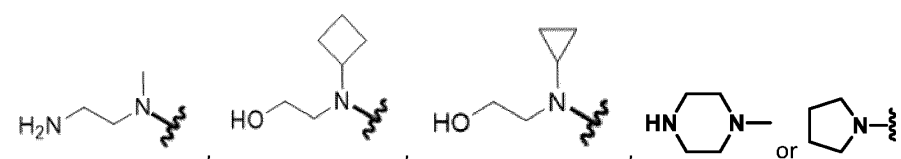
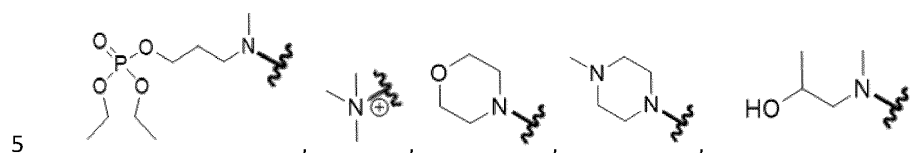
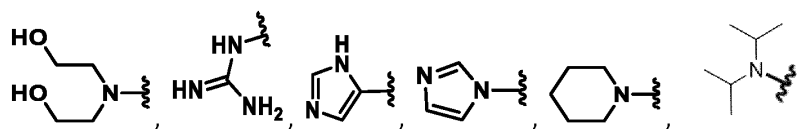
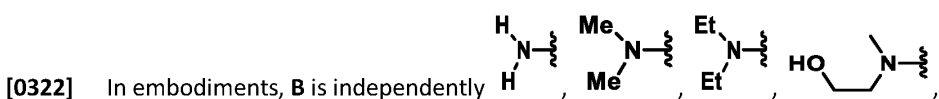
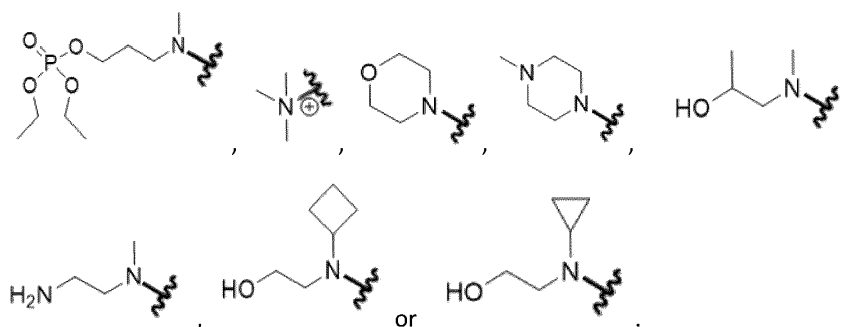
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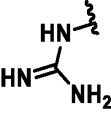
[0319] In embodiments, **B** is independently , .

[0320] In embodiments, **B** is independently , .

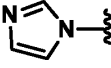
[0321] In embodiments, **B** is independently , .

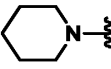
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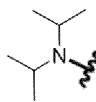


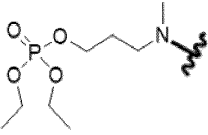
[0328] In embodiments, **B** is independently 

[0329] In embodiments, **B** is independently 

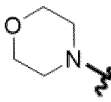
[0330] In embodiments, **B** is independently 

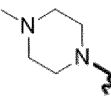
[0331] In embodiments, **B** is independently 

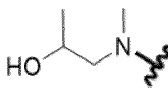
5 [0332] In embodiments, **B** is independently 

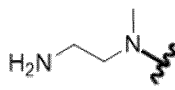
[0333] In embodiments, **B** is independently 

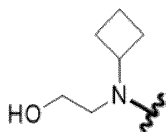
[0334] In embodiments, **B** is independently 

[0335] In embodiments, **B** is independently 

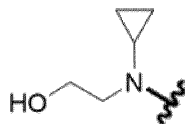
[0336] In embodiments, **B** is independently 

10 [0337] In embodiments, **B** is independently 

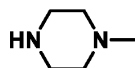
[0338] In embodiments, **B** is independently 



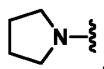
[0339] In embodiments, **B** is independently



[0340] In embodiments, **B** is independently



[0341] In embodiments, **B** is independently

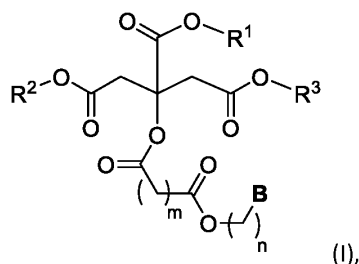


[0342] In embodiments, **B** is independently

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Formula (I)

[0343] In embodiments, a compound has a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof, wherein each of **R**¹, **R**², **R**³, **B**, **m**, and **n** is

10 independently according to any combination of embodiments described herein.

[0344] In embodiments,

each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

m is an integer of 2-10;

15 **n** is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group.

[0345] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkyl.

[0346] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkenyl.

[0347] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkynyl.

[0348] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

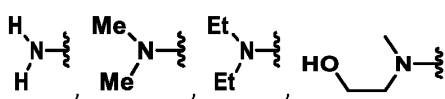
[0349] In embodiments, **B** is independently an ionizable nitrogen containing group.

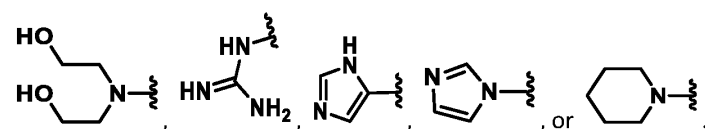
[0350] In embodiments, **B** is independently a permanently charged nitrogen containing group.

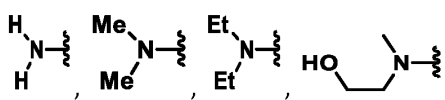
[0351] In embodiments, m is 2 and/or n is 2 or 3.

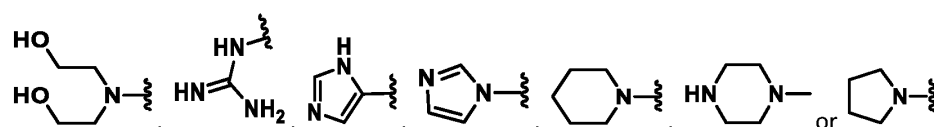
[0352] In embodiments, m is 2 and n is 2.

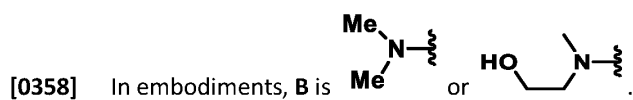
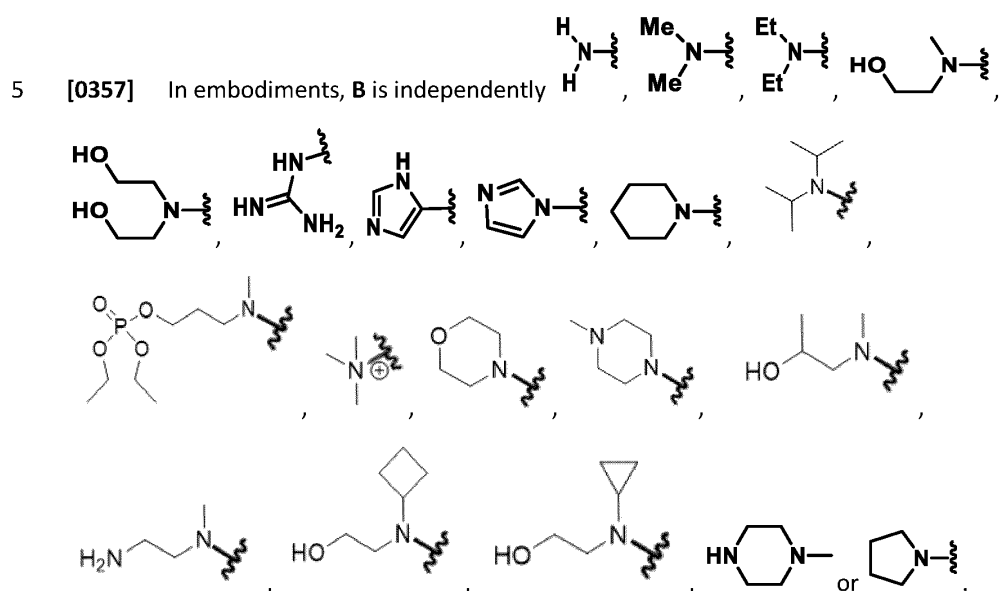
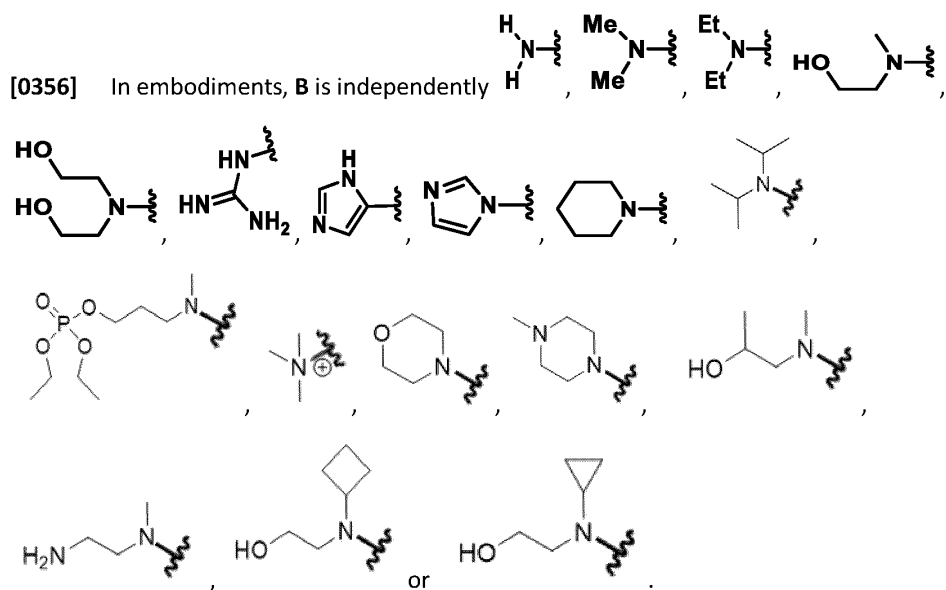
[0353] In embodiments, m is 2 and n is 3.

[0354] In embodiments, **B** is independently 

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[0355] In embodiments, **B** is independently 

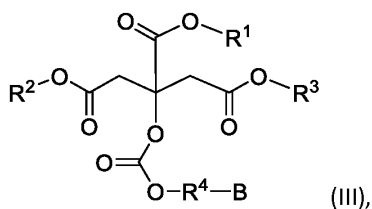




[0361] In embodiments, a compound is selected from the group consisting of: Compounds (1), (2), (3), (4), (6), (9), (17), (24), (26), (27), (33), and (35).

Formula (III)

5 [0362] In embodiments, a compound has a structure according to Formula (III):



or a pharmaceutically acceptable salt thereof, wherein each of R^1 , R^2 , R^3 , R^4 , and B is independently according to any combination of embodiments described herein.

[0363] In embodiments,

10 each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, C_6 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

R^4 is C_2 - C_{10} alkylene; and

B is independently an ionizable nitrogen-containing group.

[0364] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl. In embodiments,
15 each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkyl.

[0365] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkenyl.

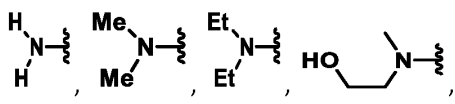
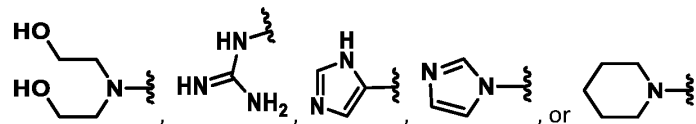
20 [0366] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkynyl.

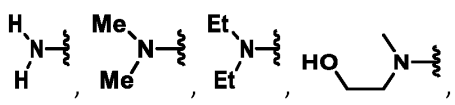
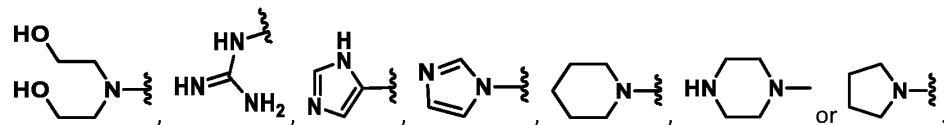
[0367] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30}
25 heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

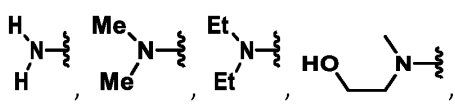
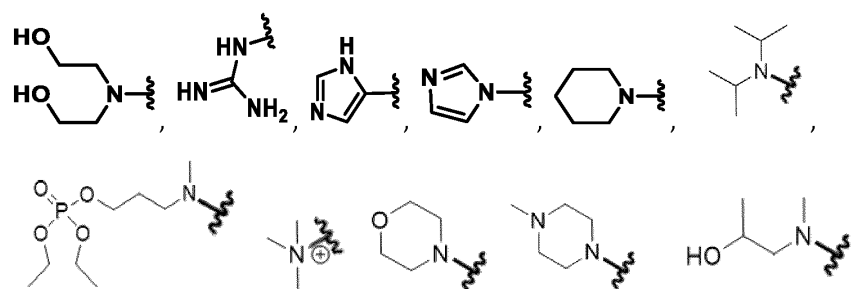
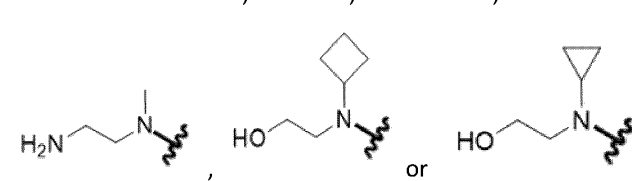
[0368] In embodiments, R^4 is unsubstituted C_2 - C_{10} alkylene.

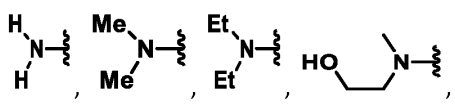
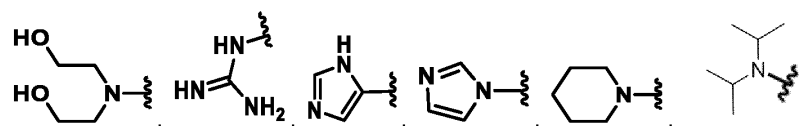
[0369] In embodiments, R^4 is substituted C_2 - C_{10} alkylene.

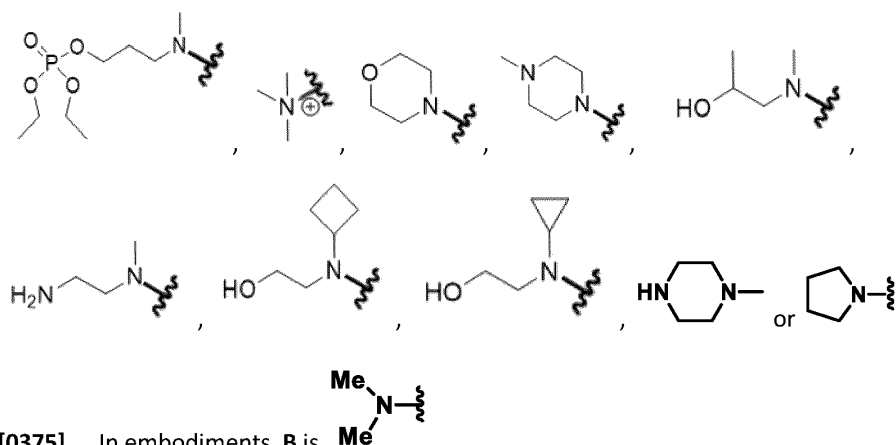
[0370] In embodiments, R^4 is $-CH_2CH_2-$.

[0371] In embodiments, **B** is independently , .

5 [0372] In embodiments, **B** is independently , .

[0373] In embodiments, **B** is independently , , .

[0374] In embodiments, **B** is independently , .



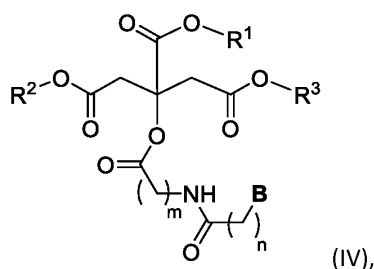
[0375] In embodiments, **B** is

[0376] In embodiments, a compound is Compound (3).

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Formula (IV)

[0377] In embodiments, a compound has a structure according to Formula (IV):



or a pharmaceutically acceptable salt thereof, wherein each of R^1 , R^2 , R^3 , m , n , and **B** is

10 independently according to any combination of embodiments described herein.

[0378] In embodiments,

each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, C_6 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

m is an integer of 2-10;

15 n is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group.

[0379] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkyl.

[0380] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkenyl.

[0381] In embodiments, each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_6 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_6 - C_{30} alkynyl.

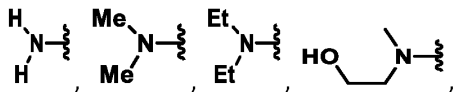
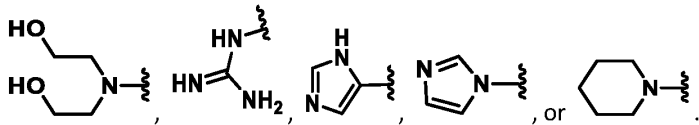
[0382] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

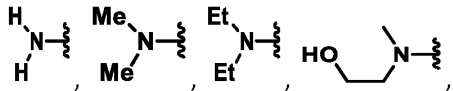
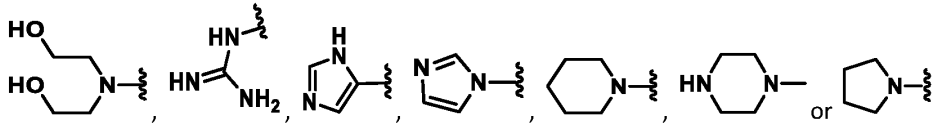
[0383] In embodiments, R^1 , R^2 , and R^3 is C_4 - C_{30} heteroalkyl.

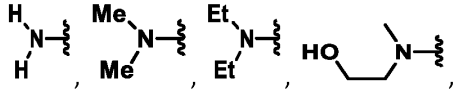
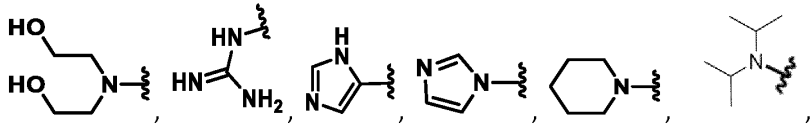
[0384] In embodiments, m is 2 and/or n is 2 or 3.

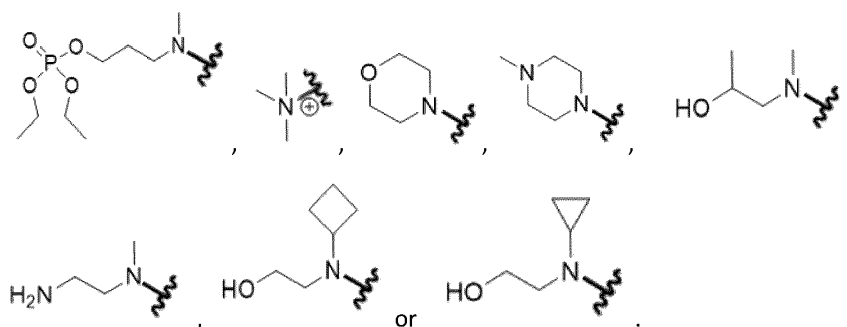
[0385] In embodiments, m is 2 and n is 2.

[0386] In embodiments, m is 2 and n is 3.

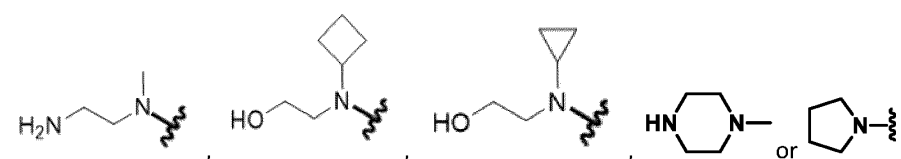
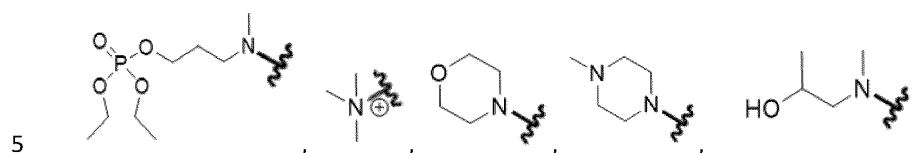
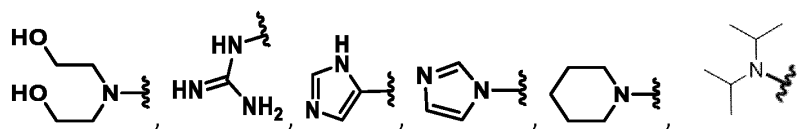
[0387] In embodiments, B is independently , .

[0388] In embodiments, B is independently , .

[0389] In embodiments, B is independently , .



[0390] In embodiments, **B** is independently NC, CN(C)C, CCN(C)C, CC(C)C(C)N(C)C,

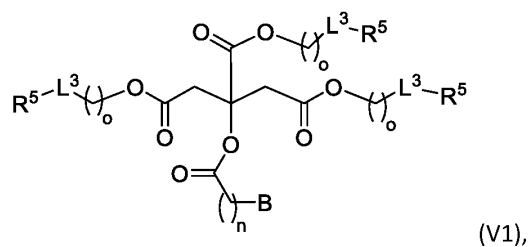


[0391] In embodiments, **B** is CN(C)C.

[0392] In embodiments, a compound is Compound (8) or (39).

Formula (V1)

10 [0393] In embodiments, a compound has a structure according to Formula (V1):



or a pharmaceutically acceptable salt thereof, wherein each of R^5 , L^3 , n , o , and B is independently according to any combination of embodiments described herein.

[0394] In embodiments,

n is an integer of 2, 3, 4, 5, 6, or 7; and

5 B is independently an ionizable nitrogen-containing group.

[0395] In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl.

[0396] In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_6 alkyl.

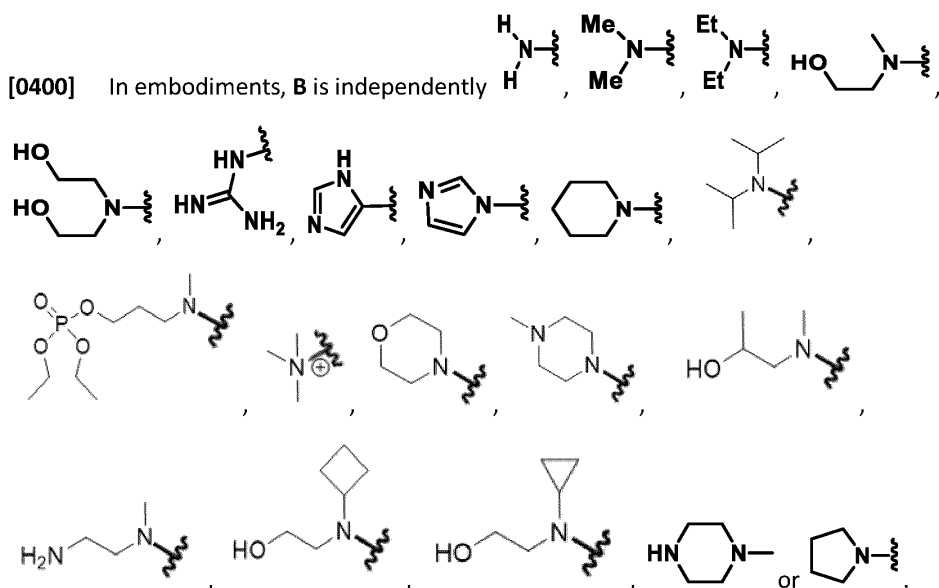
[0397] In embodiments, B is independently

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[0398] In embodiments, B is independently

[0399] In embodiments, B is independently

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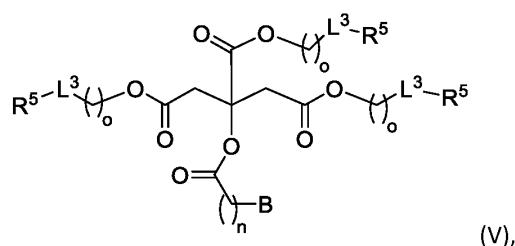


- 5 [0401] In embodiments, **B** is
-

[0402] In embodiments, a compound is selected from the group consisting of: Compounds (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), (40), (112), (113), (114), (115), (116), (117), (126), (127), (128), and (129).

Formula (V)

- 10 [0403] In embodiments, a compound has a structure according to Formula (V):



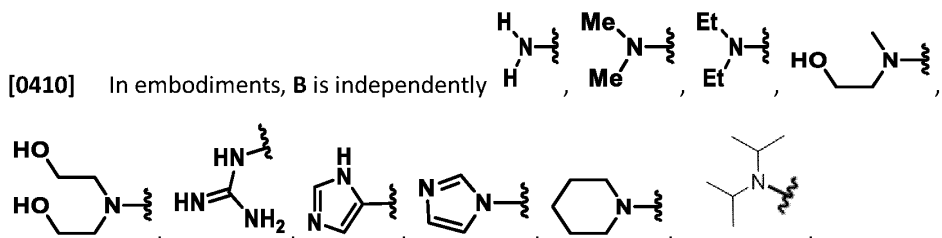
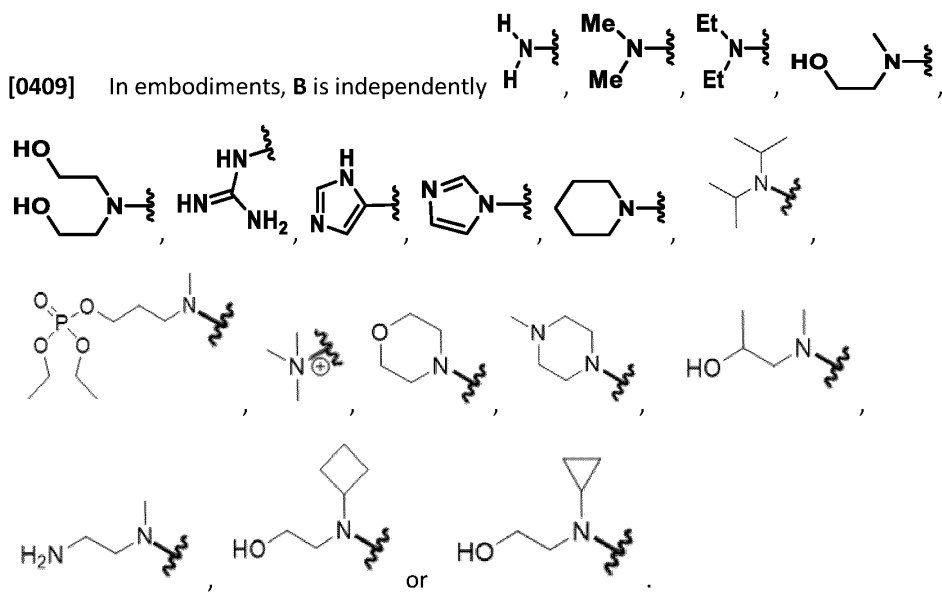
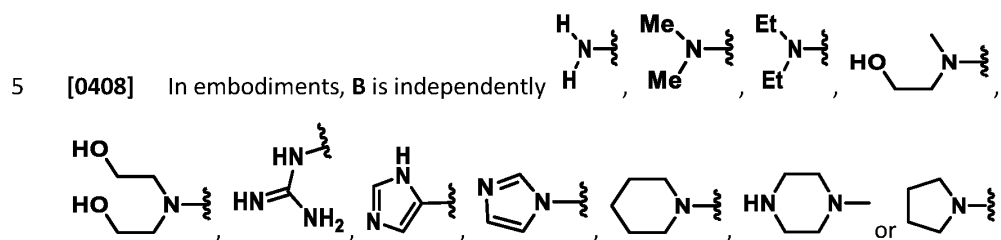
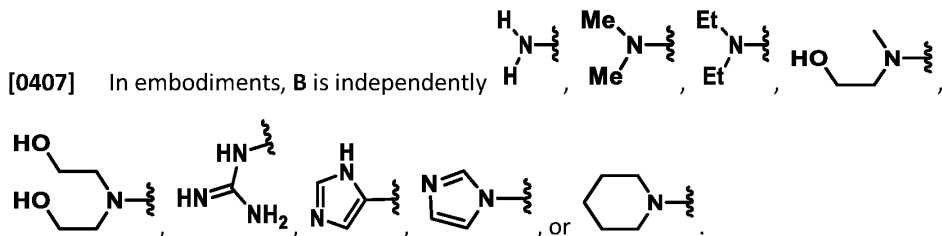
or a pharmaceutically acceptable salt thereof, wherein each of R^5 , L^3 , n , o , and **B** is independently according to any combination of embodiments described herein.

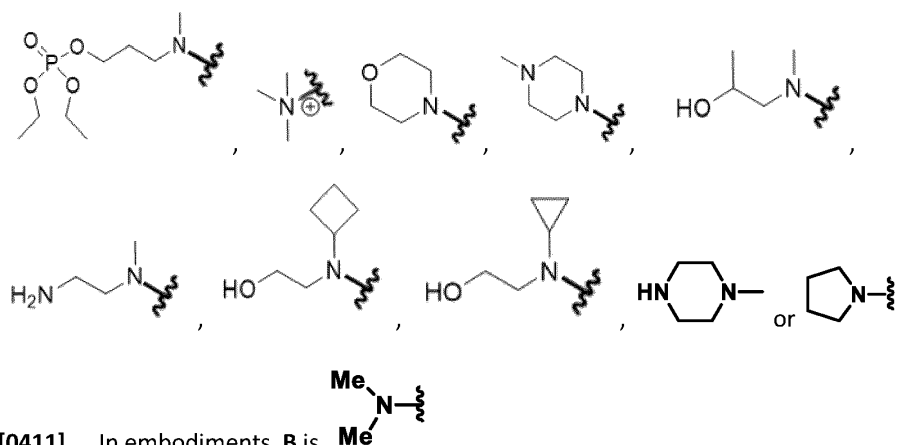
[0404] In embodiments,

- 15 **n** is an integer of 2, 3, or 4; and
B is independently an ionizable nitrogen-containing group.

[0405] In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; α is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl.

[0406] In embodiments, L^3 is $OC(O)$, CO_2 , or $(O)CO$; α is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.





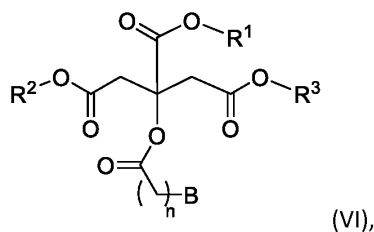
[0411] In embodiments, **B** is .

[0412] In embodiments, a compound is selected from the group consisting of: Compounds
5 (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), (40), (114), and (116).

[0413] In embodiments, a compound is selected from the group consisting of: Compounds
(11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), and (40).

Formula (VI)

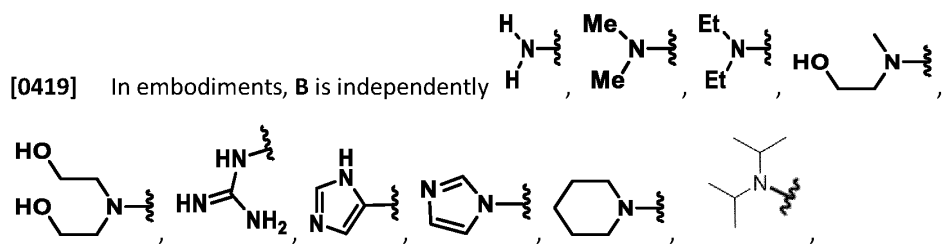
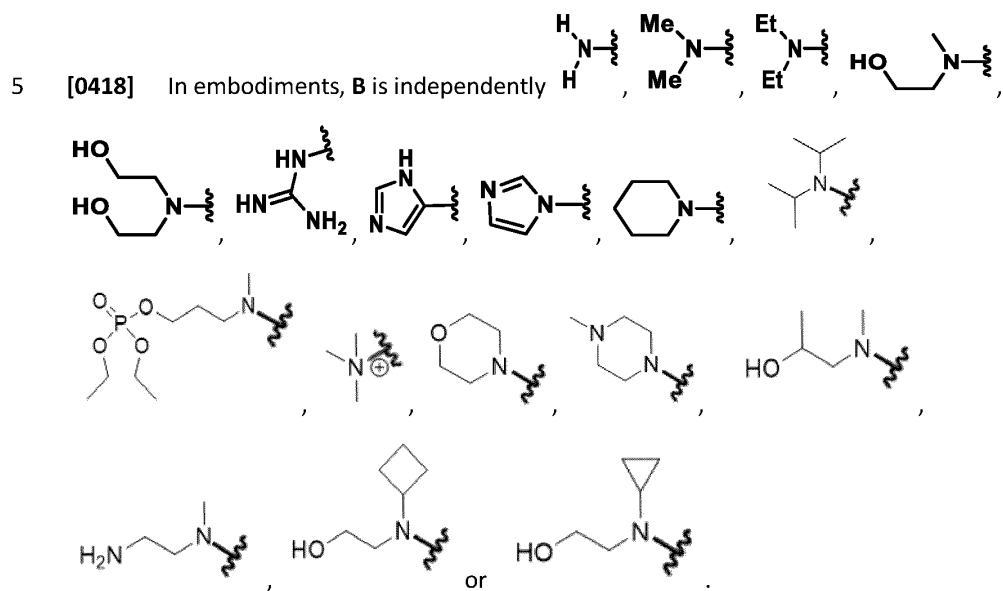
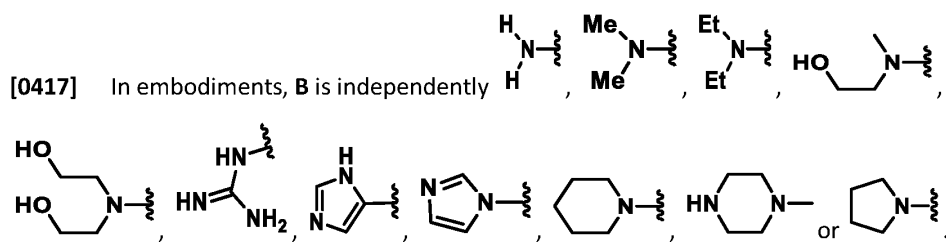
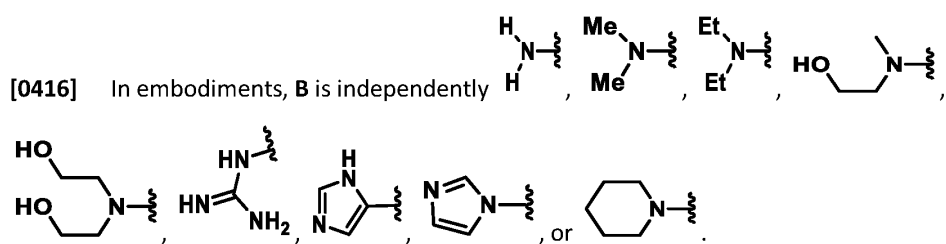
10 [0414] In embodiments, a compound has a structure according to Formula (VI):

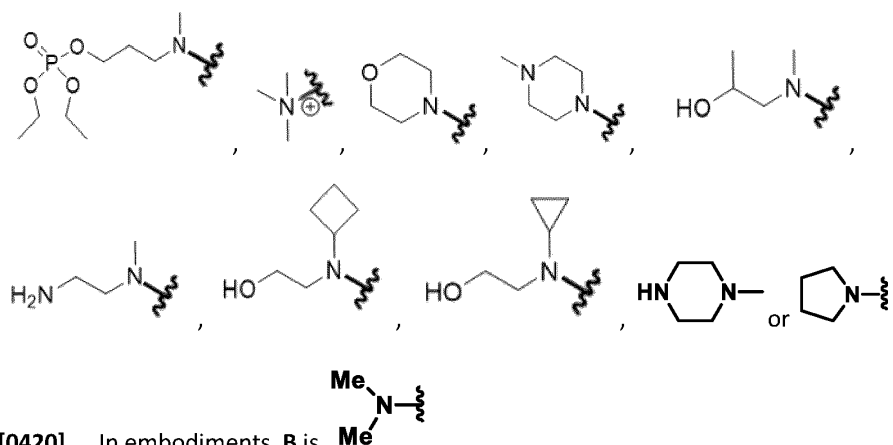


or a pharmaceutically acceptable salt thereof, wherein each of R^1 , R^2 , R^3 , n , and **B** is independently according to any combination of embodiments described herein.

[0415] In embodiments,

15 each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} heteroalkyl comprising a disulfide group;
 n is an integer of 2, 3, or 4; and
B is independently an ionizable nitrogen-containing group.



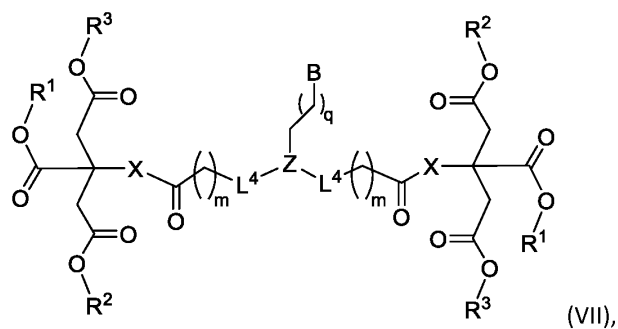


[0421] In embodiments, a compound is Compound (21).

5

Formula (VII)

[0422] In one aspect, a cationic lipid has a formula according to Formula (VII),



or a pharmaceutically acceptable salt thereof, wherein

- 10 **X** is independently O or NH;
 each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;
 each **m** is an integer of 2-10;
 each **L⁴** is a carbonyl, ester, or amide;
- 15 **Z** is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, wherein **q1** and **q2** are independently integers of 2-10; or **Z** is C₆H₅-Z¹, wherein **Z¹** is a carbonyl, ester, or amide that is covalently attached to the $-CH_2(CH_2)_qB$ moiety;
 q is an integer of 1-9; and
 B is independently an ionizable nitrogen-containing group.

[0423] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkyl.

[0424] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkenyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkenyl.

[0425] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} alkynyl. In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} alkynyl.

[0426] In embodiments, each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} heteroalkyl (e.g., C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently unsubstituted C_4 - C_{30} heteroalkyl (e.g., unsubstituted C_6 - C_{30} heteroalkyl). In embodiments, each of R^1 , R^2 , and R^3 is independently substituted C_4 - C_{30} heteroalkyl (e.g., substituted C_6 - C_{30} heteroalkyl).

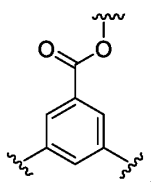
[0427] In embodiments, L^4 is a carbonyl.

[0428] In embodiments, L^4 is an ester. In embodiments, L^4 is $-OC(O)-$. In embodiments, L^4 is $-(O)CO-$.

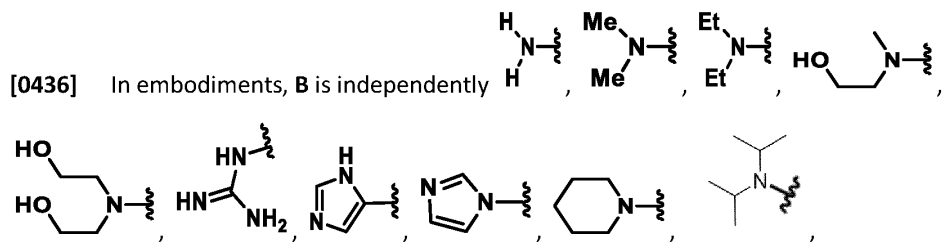
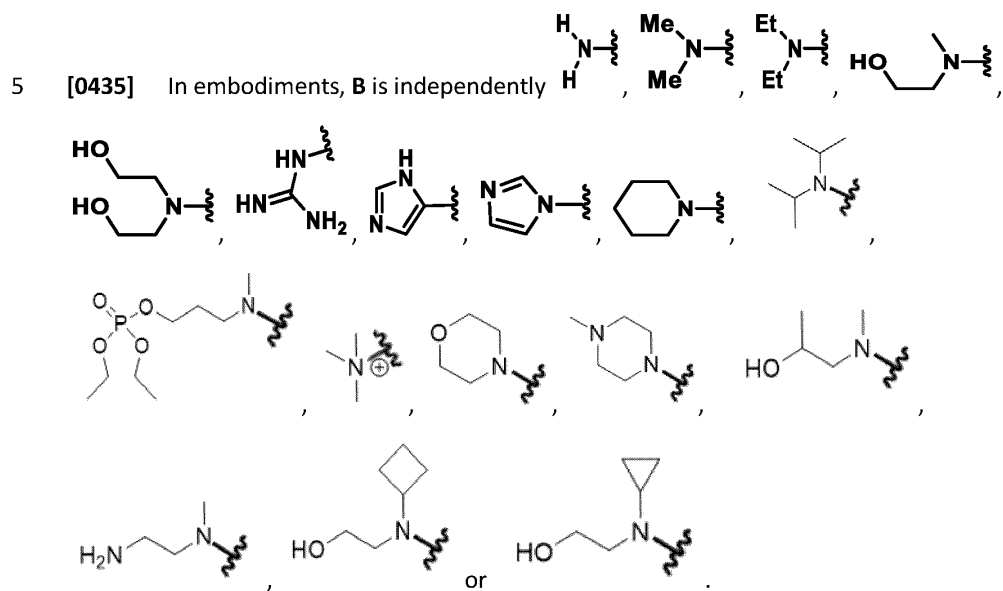
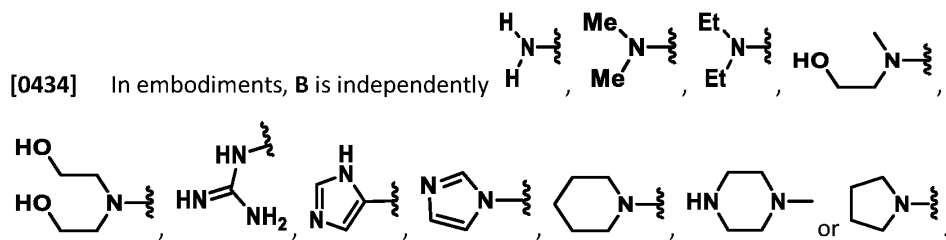
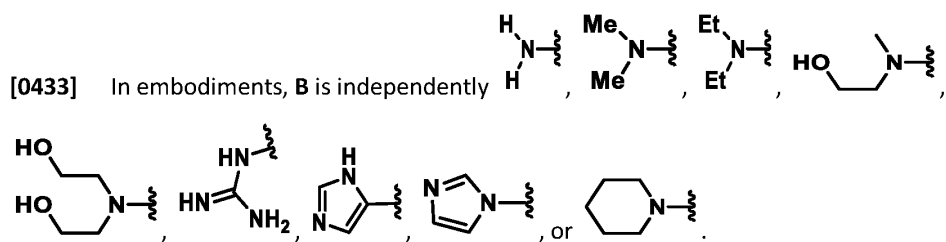
[0429] In embodiments, L^4 is an amide. In embodiments, L^4 is $-NHC(O)-$. In embodiments, L^4 is $-C(O)NH-$.

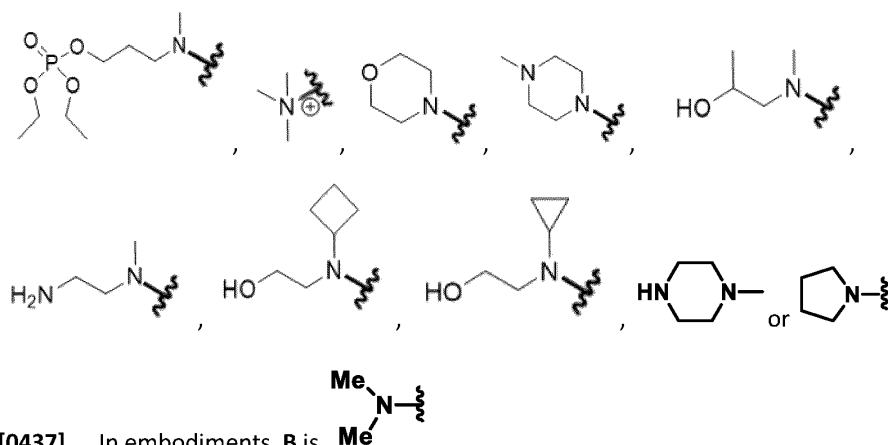
[0430] In embodiments, X is O; each L^4 is $-C(O)O-$; m is an integer of 2, 3, or 4; and/or q is 1.

[0431] In embodiments, Z is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, and $q1$ and $q2$ are each 2.



[0432] In embodiments, Z is .





[0438] In embodiments, a compound is Compound (16) or (31).

- 5 [0439] In one aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound
- 10 having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof.

[0440] In one aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according

15 to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof.

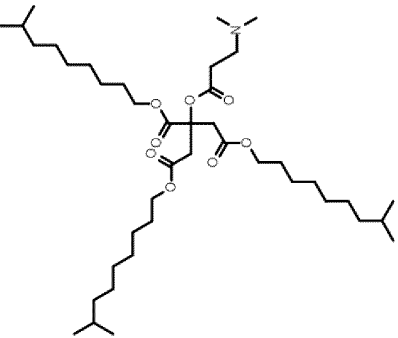
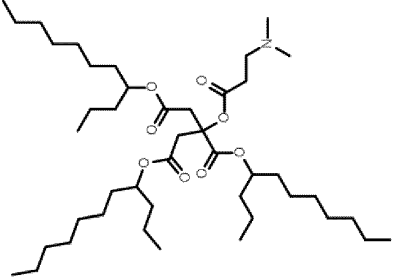
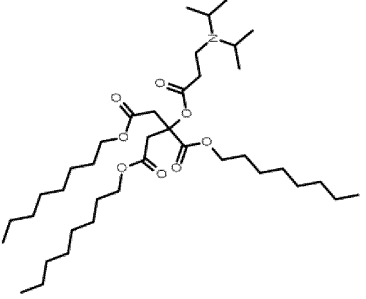
[0441] In one aspect, the invention features a method of intranasal delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids,

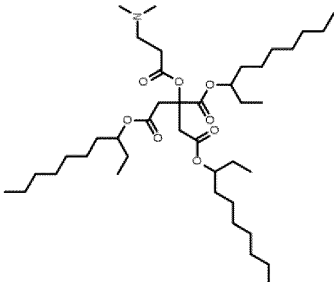
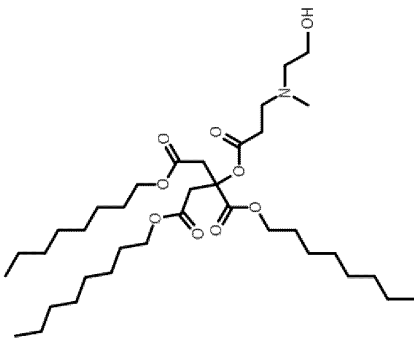
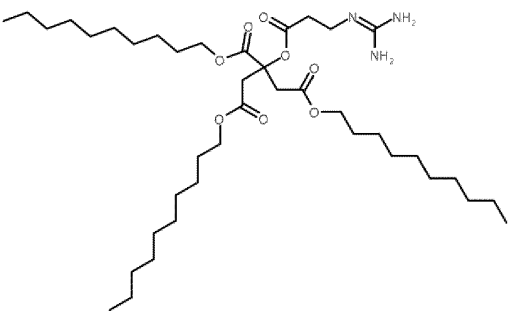
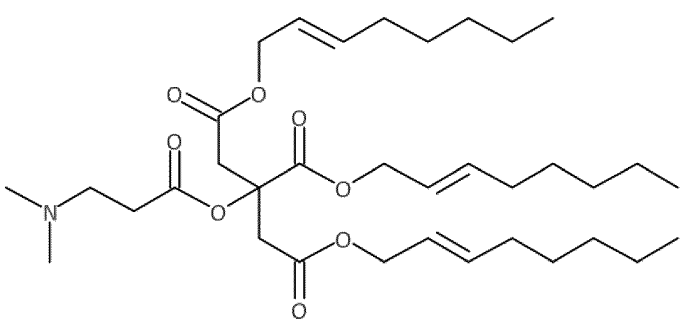
20 optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof, for example Compound 64, or a compound in Table 6, or a pharmaceutically acceptable salt thereof.

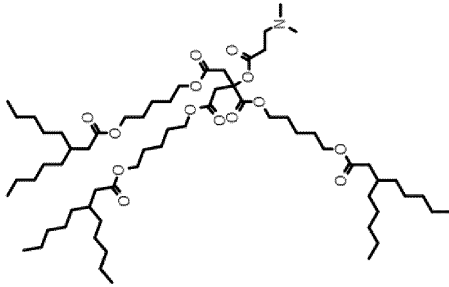
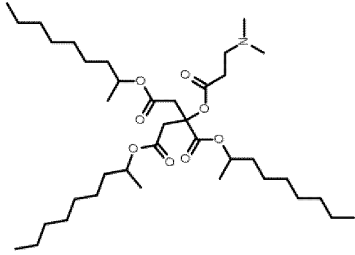
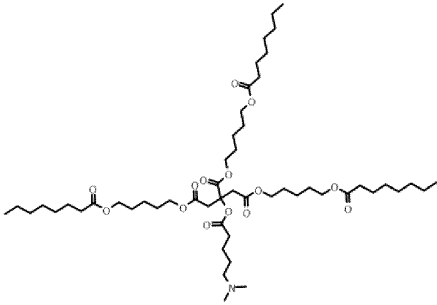
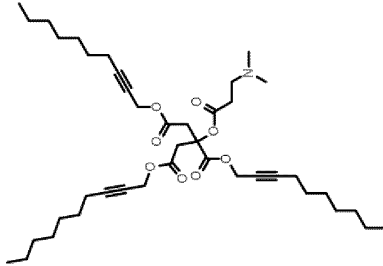
- [0442] In one aspect, the invention features a method of intranasal delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (A2), Formula (A1) or Formula (A) as described herein, or a pharmaceutically acceptable salt thereof, for example
5 Compound 64, or a compound in Table 6, or a pharmaceutically acceptable salt thereof.
- [0443] Intranasal administration includes administration via the nose, either with or without concomitant inhalation during administration. Such administration is typically through contact by the composition with the nasal mucosa, nasal turbinates or sinus cavity. The pharmaceutical compositions for administration may be applied in a single administration or in multiple
10 administrations. For example, one dose can be placed in each nostril during administration. For example, bi-dose delivery can be used with the compositions according to the invention. Bi-dose devices contain two sub-doses of a single dose, one sub-dose for administration to each nostril. Generally, the two sub-doses are present in a single chamber and the construction of the device allows the efficient delivery of a single sub-dose at a time. Alternatively, a mono-dose device may
15 be used for administering the compositions according to the invention. The composition can be given in one, two, three, four, or more doses, so that the subject is given a first dose (which can be a bi-dose or mono-dose, as described above), and then a second dose is administered within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days, or 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks, or 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or 1, 2, 3, 4,
20 5, 6, 7, 8, 9, or 10 or more years apart. Exemplary devices for intranasal administration of the compositions according to the invention are spray devices. Suitable commercially available nasal spray devices include Accuspray™ (Becton Dickinson). Nebulizers produce a very fine spray (such as a mist) which can be easily inhaled and are also contemplated herein. Exemplary spray devices for intranasal use are devices for which the performance of the device is not dependent upon the
25 pressure applied by the user. These devices are known as pressure threshold devices. Liquid is released from the nozzle only when a threshold pressure is applied. These devices make it easier to achieve a spray with a regular droplet size. Pressure threshold devices suitable for use with the present invention are known in the art.
- [0444] The invention provides in a further aspect a pharmaceutical kit comprising an intranasal
30 administration device as described herein containing a formulation according to the invention.
- [0445] The invention is not necessarily limited to spray delivery of liquid formulations. Compositions according to the invention may be administered in other forms e.g. as a powder.

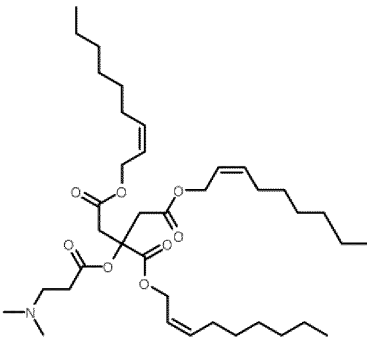
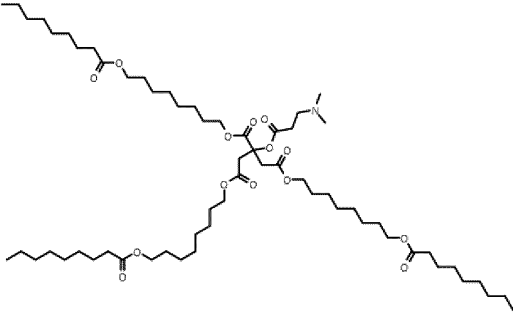
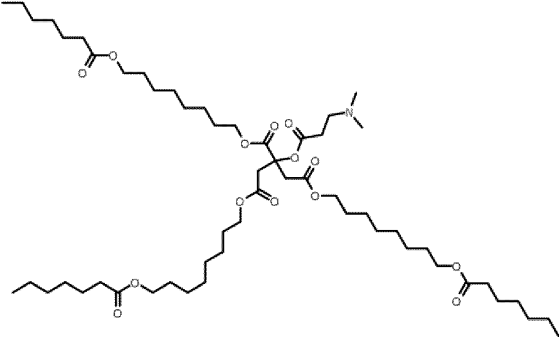
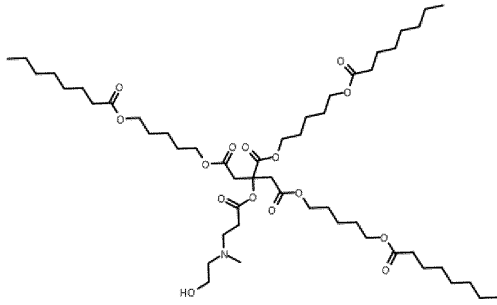
Compounds 41-64 and 90

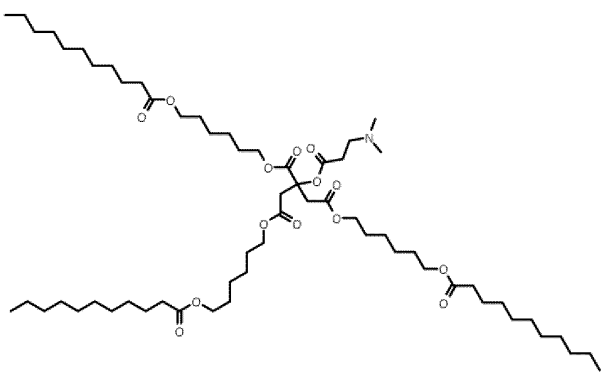
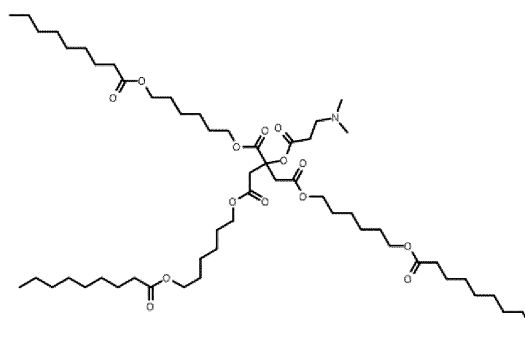
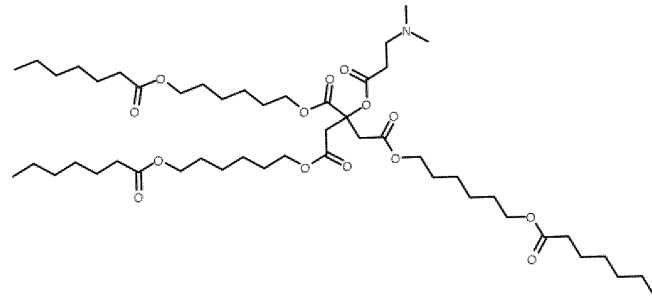
[0446] In another aspect, a cationic lipid is selected from the group of Compounds 41-64 and 90, or a pharmaceutically acceptable salt thereof:

No.	Structure
41	
42	
43	

No.	Structure
44	
45	
46	
47	

No.	Structure
48	
49	
50	
51	

No.	Structure
52	
53	
54	
55	

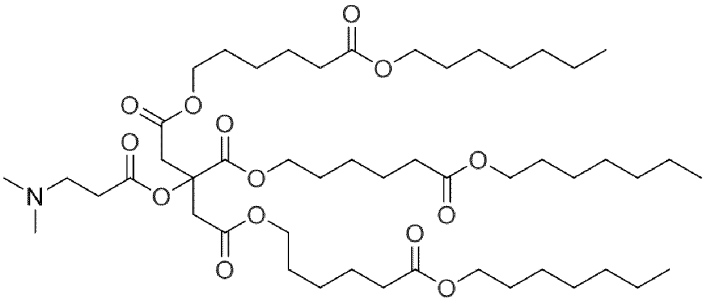
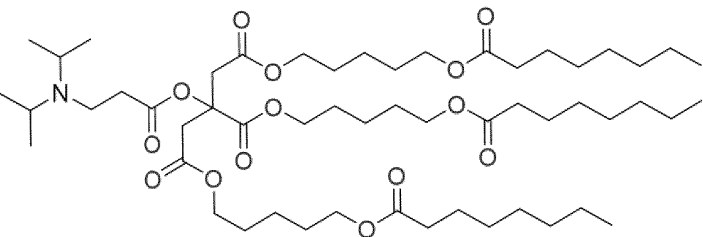
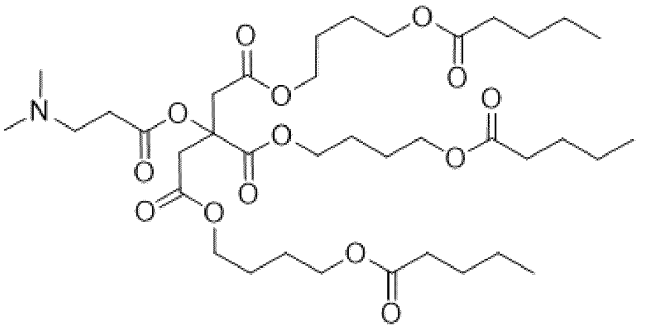
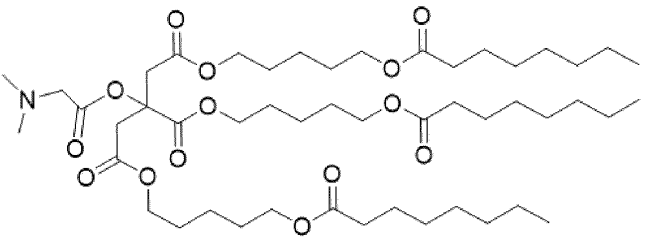
No.	Structure
56	
57	
58	

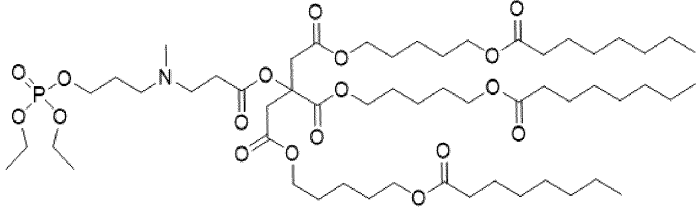
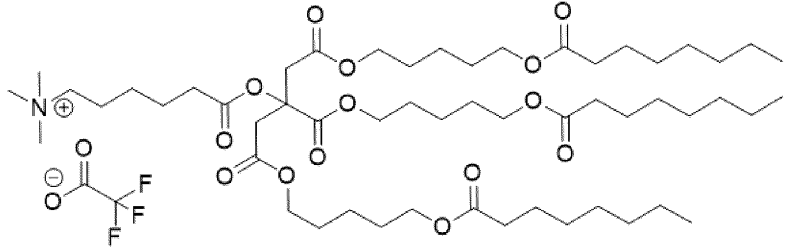
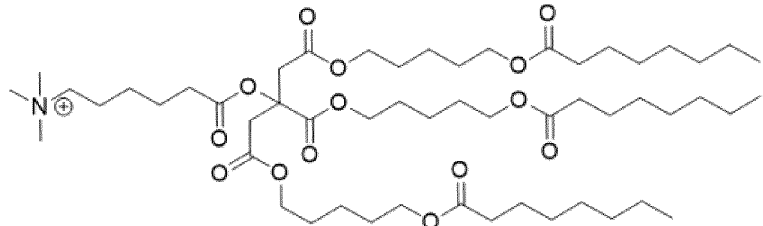
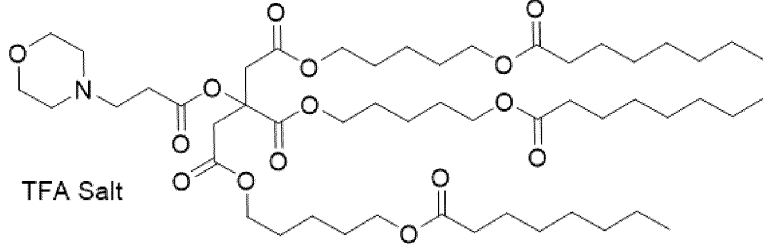
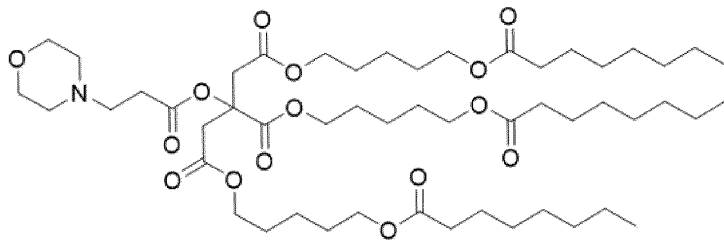
No.	Structure
59	
60	
61	
62	

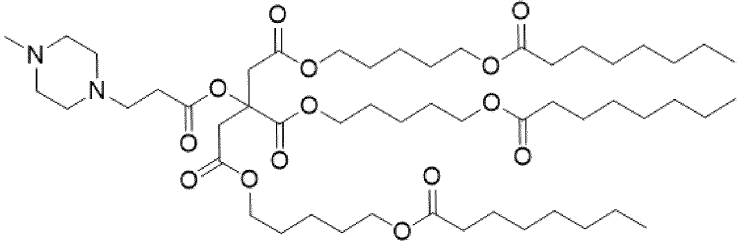
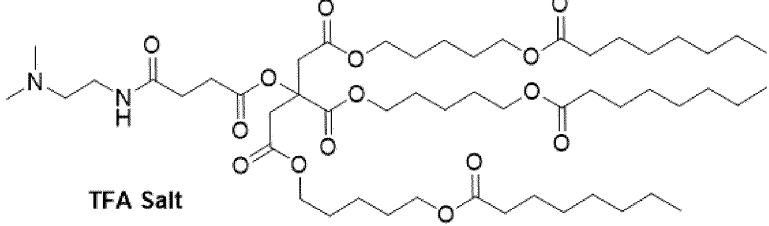
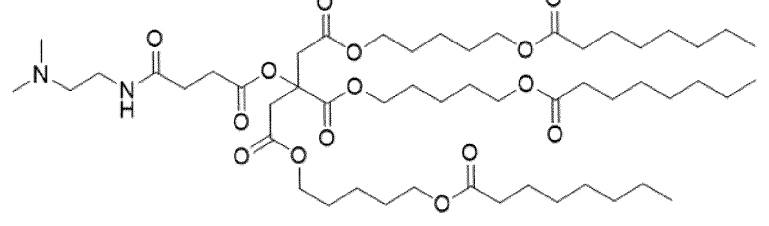
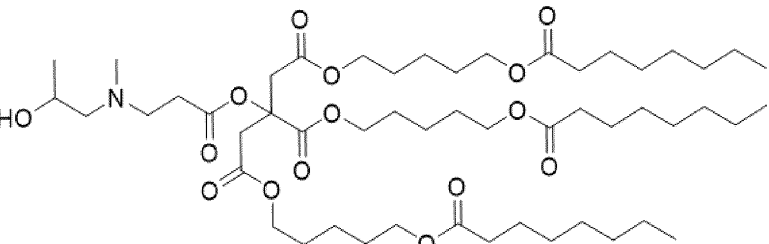
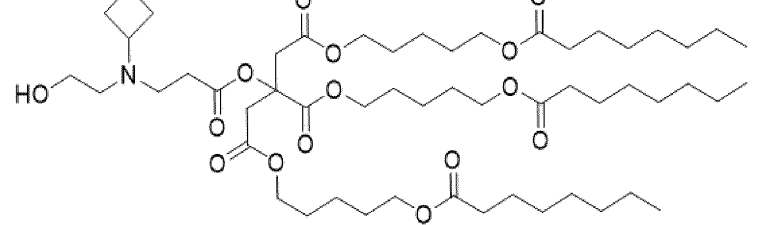
No.	Structure
63	
64	
90	

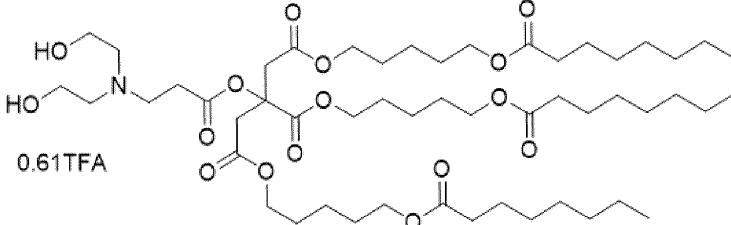
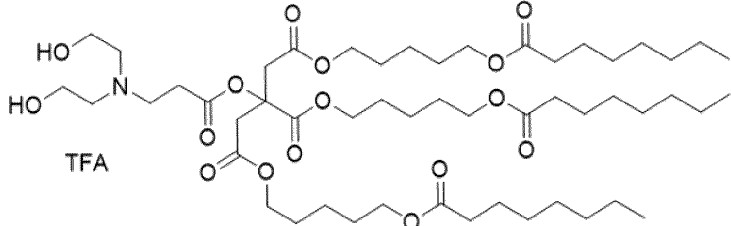
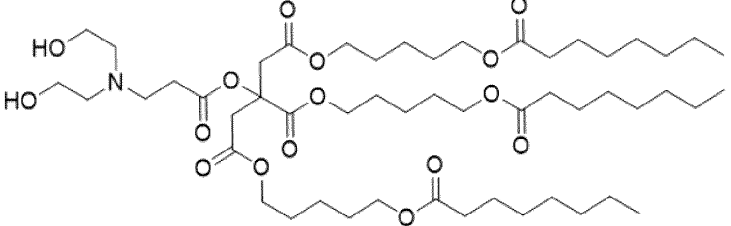
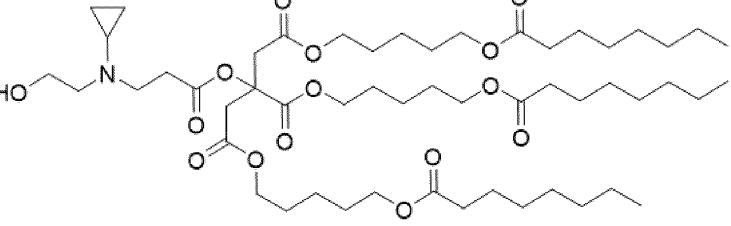
Compounds 91-111

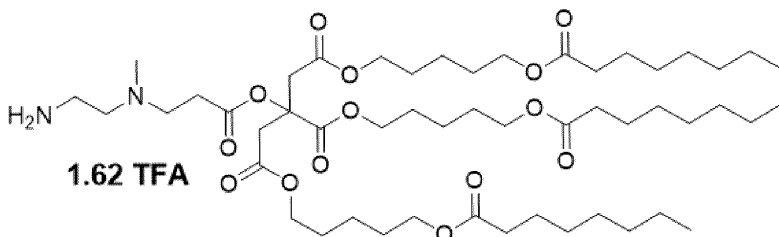
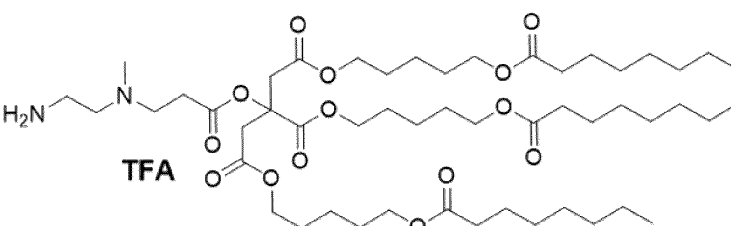
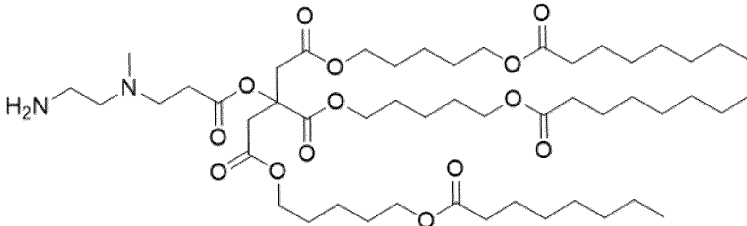
[0447] In another aspect, a cationic lipid is selected from the group of Compounds 91-111 or a pharmaceutically acceptable salt thereof:

No.	Structure
91	
92	
93	
94	

No.	Structure
95	
96	
97	
98	 TFA Salt
99	

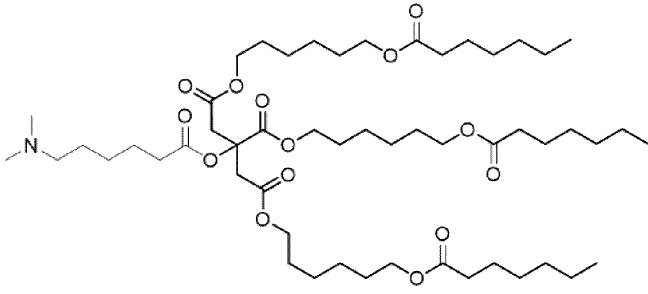
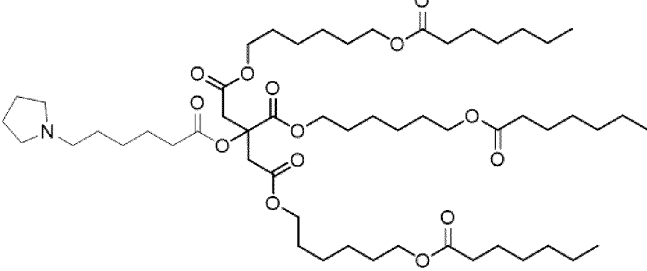
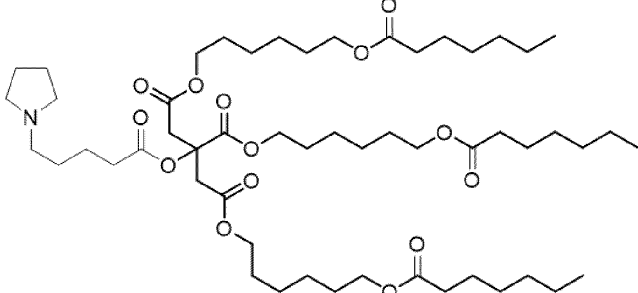
No.	Structure
100	
101	 TFA Salt
102	
103	
104	

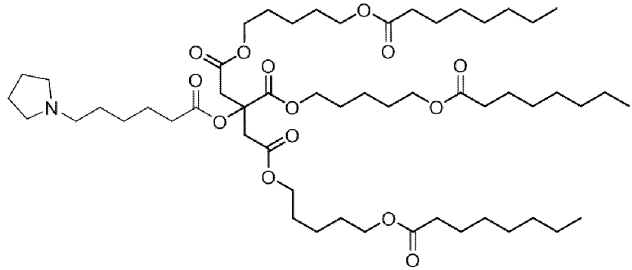
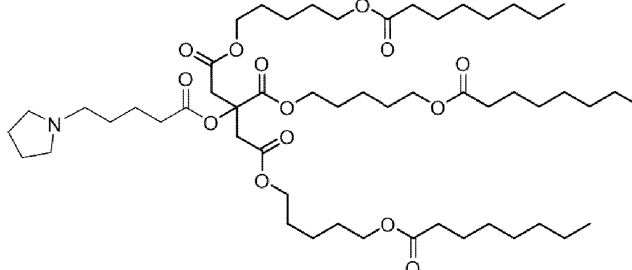
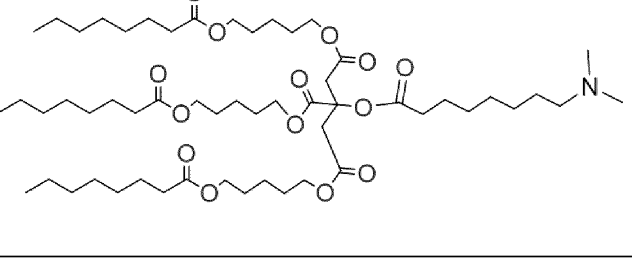
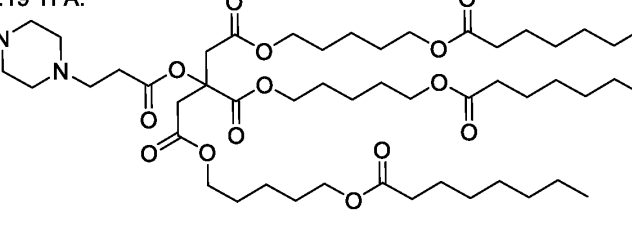
No.	Structure
105	 0.61TFA
106	 TFA
107	
108	

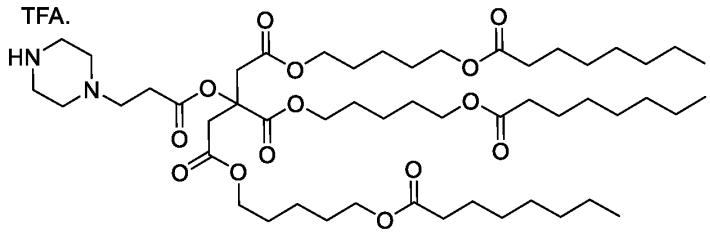
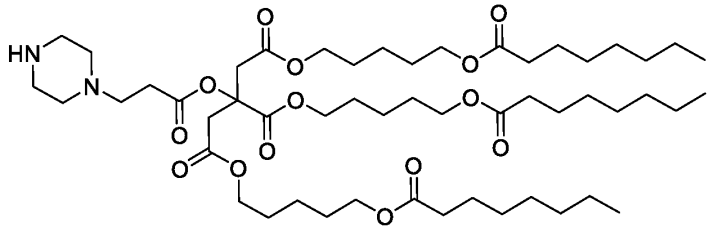
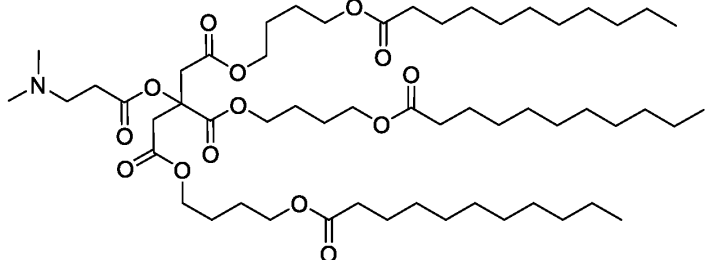
No.	Structure
109	 1.62 TFA TFA content 15% (w/w)
110	 TFA
111	

Compounds 112-117

[0448] In another aspect, a cationic lipid is selected from the group of Compounds 112-117 and 126-129, or a pharmaceutically acceptable salt thereof:

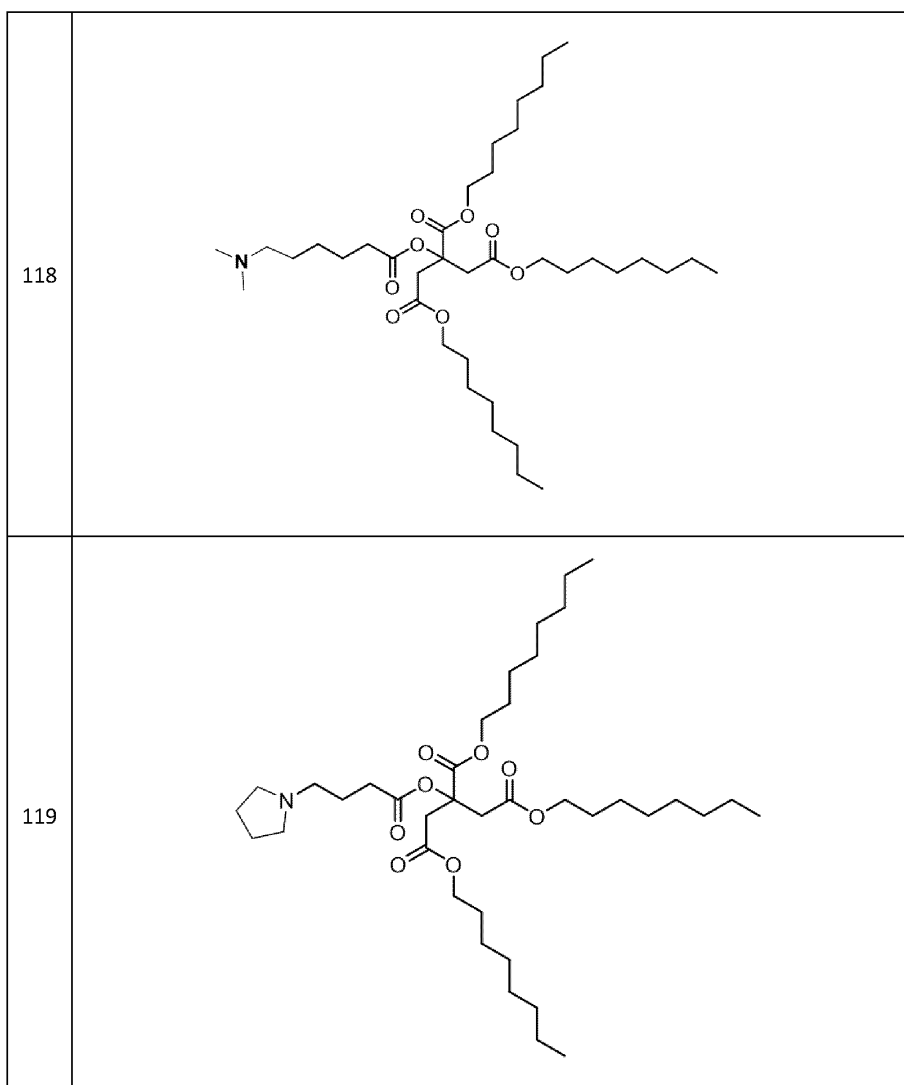
No.	Structure
112	
113	
114	

No.	Structure
115	
116	
117	
126	3.19 TFA. 

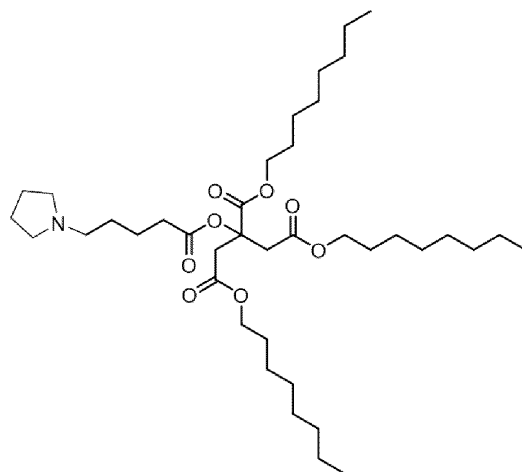
No.	Structure
127	
128	
129	

Compounds 118-125

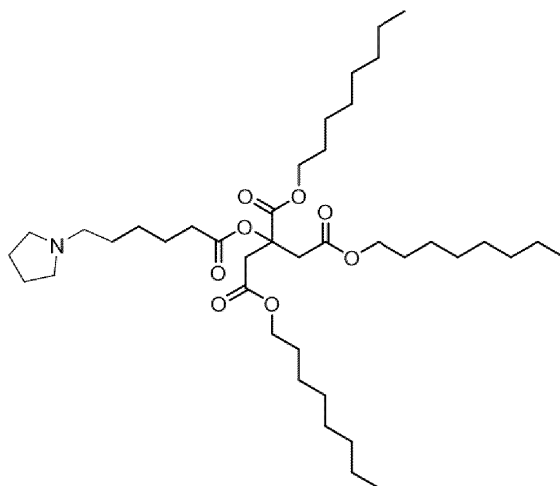
[0449] In another aspect, a cationic lipid is selected from the group of Compounds 118-125 or a pharmaceutically acceptable salt thereof:



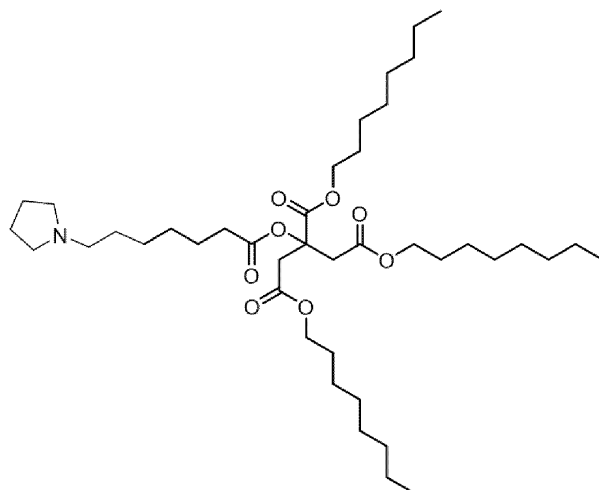
120



121



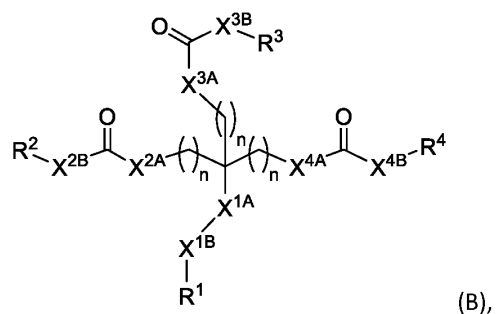
122



123	
124	
125	

Formula (B)

[0450] In another aspect, the invention features a cationic lipid according to Formula (B).



5 or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A};

R^{1A} is H or C₁-C₆ alkyl;

X^{1B} is a covalent bond, C(O), CH₂CO₂, or CH₂C(O);

10 one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R^1 is independently L^1-B^1 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

R^2 is independently L^2-B^2 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

5 R^3 is independently L^3-B^3 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

R^4 is independently L^4-B^4 , C_6-C_{30} alkyl, C_6-C_{30} alkenyl, or C_6-C_{30} alkynyl;

L^1 , L^2 , L^3 , and L^4 are each independently C_1-C_{30} alkylene; C_2-C_{30} alkenylene; or C_2-C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

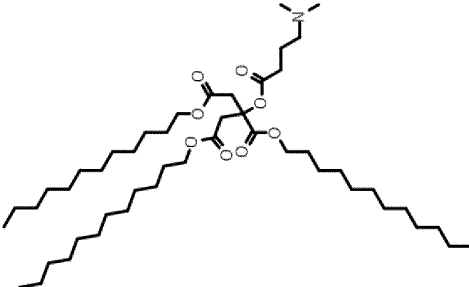
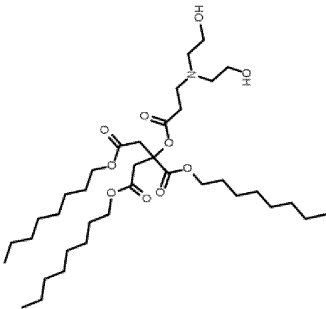
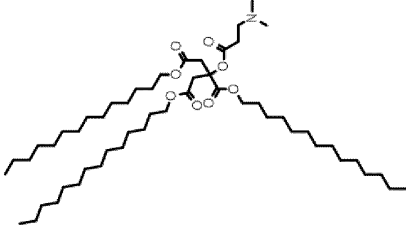
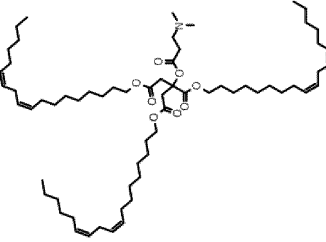
10 and

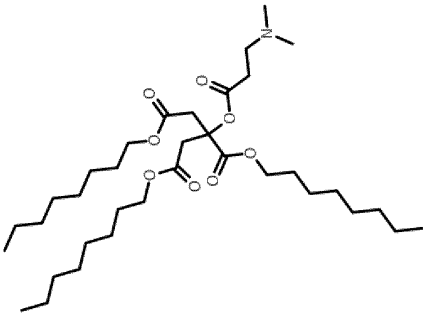
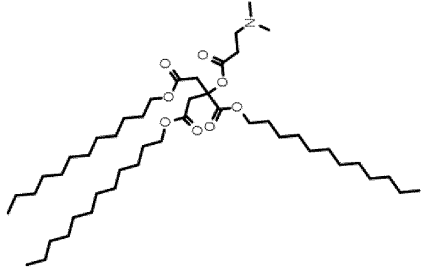
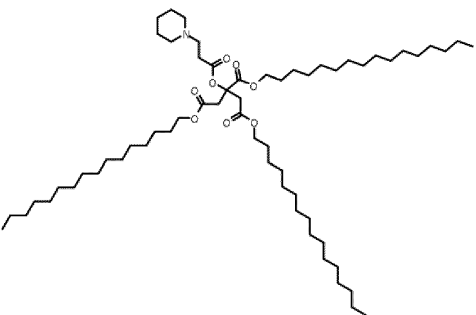
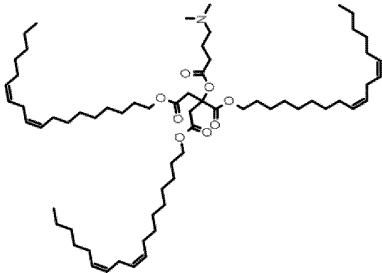
wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

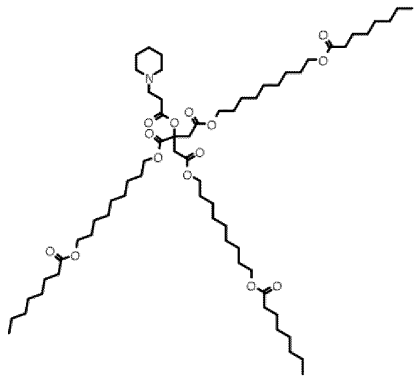
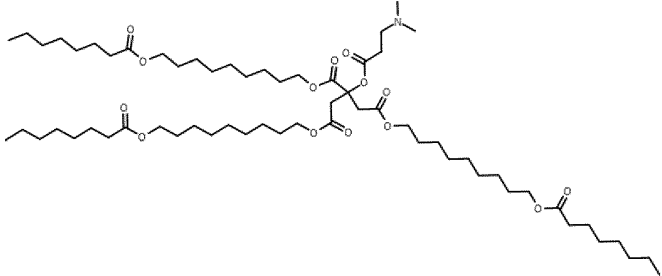
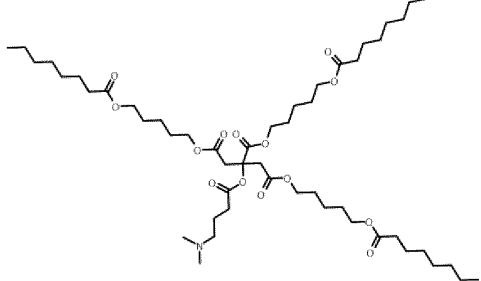
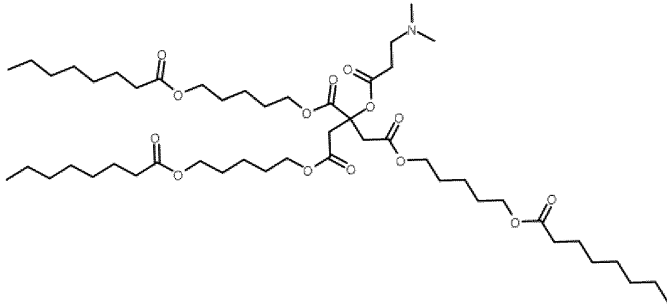
[0451] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B) as described herein, or a pharmaceutically acceptable salt thereof.

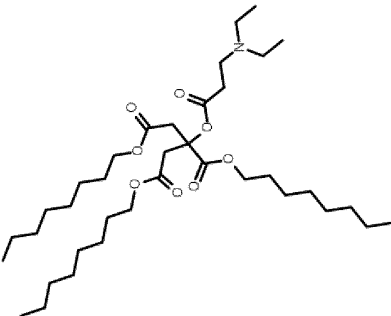
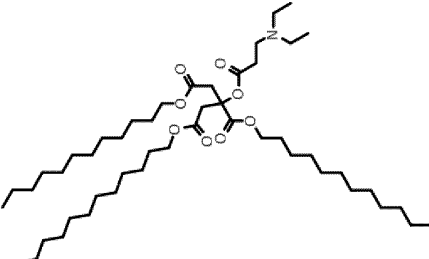
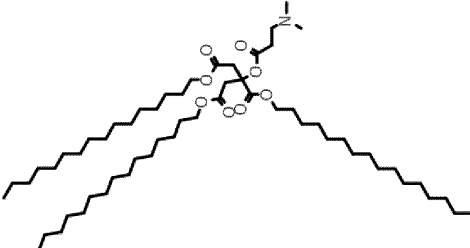
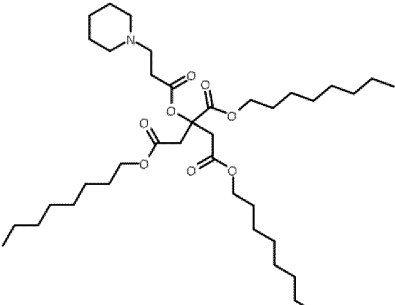
[0452] In another aspect, the invention features a method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B) as described herein, or a pharmaceutically acceptable salt thereof.

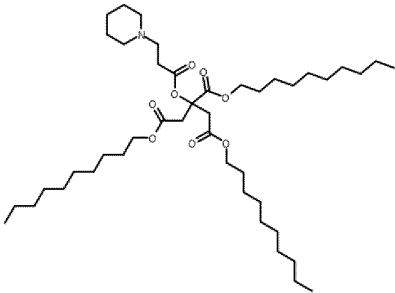
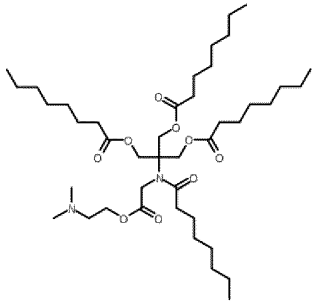
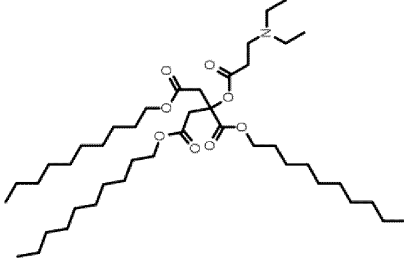
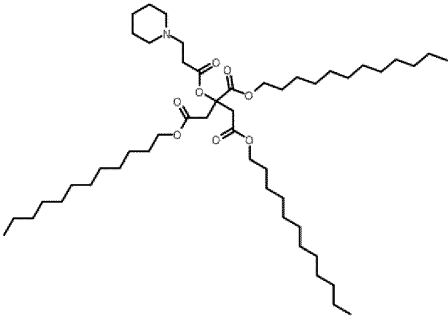
[0453] In embodiments, a compound of Formula (B) is selected from the group consisting of Compounds (65)-(89), or a pharmaceutically acceptable salt thereof:

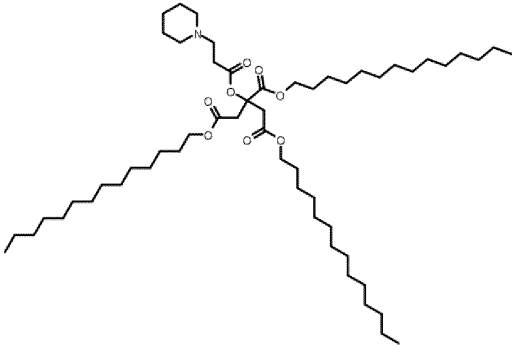
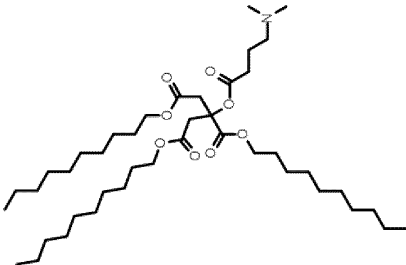
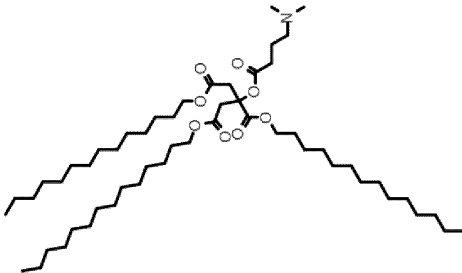
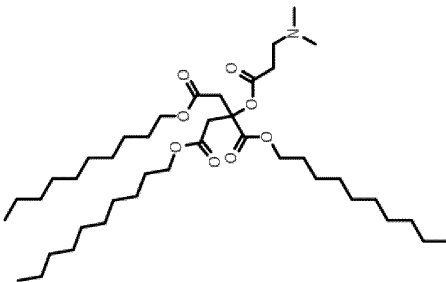
No.	Structure
65	 Chemical structure 65 is a complex molecule featuring a central core with multiple ester and ether linkages, and long alkyl chains. The structure is symmetrical, with a central core connected to four long alkyl chains via ester and ether linkages. The central core appears to be a substituted cyclohexane or similar ring system.
66	 Chemical structure 66 is a complex molecule featuring a central core with multiple ester and ether linkages, and long alkyl chains. The structure is symmetrical, with a central core connected to four long alkyl chains via ester and ether linkages. The central core appears to be a substituted cyclohexane or similar ring system.
67	 Chemical structure 67 is a complex molecule featuring a central core with multiple ester and ether linkages, and long alkyl chains. The structure is symmetrical, with a central core connected to four long alkyl chains via ester and ether linkages. The central core appears to be a substituted cyclohexane or similar ring system.
68	 Chemical structure 68 is a complex molecule featuring a central core with multiple ester and ether linkages, and long alkyl chains. The structure is symmetrical, with a central core connected to four long alkyl chains via ester and ether linkages. The central core appears to be a substituted cyclohexane or similar ring system.

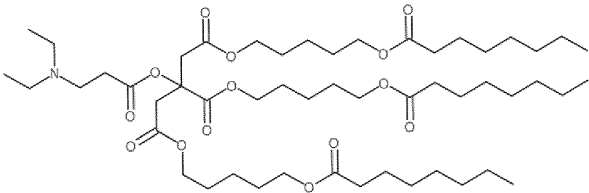
No.	Structure
69	
70	
71	
72	

No.	Structure
73	 Chemical structure 73: A central carbon atom bonded to a piperidine ring, a long alkyl chain, and two long alkyl chains with ester groups.
74	 Chemical structure 74: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains with ester groups.
75	 Chemical structure 75: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains with ester groups.
76	 Chemical structure 76: A central carbon atom bonded to a dimethylamino group, a long alkyl chain, and two long alkyl chains with ester groups.

No.	Structure
77	
78	
79	
80	

No.	Structure
81	
82	
83	
84	

No.	Structure
85	
86	
87	
88	

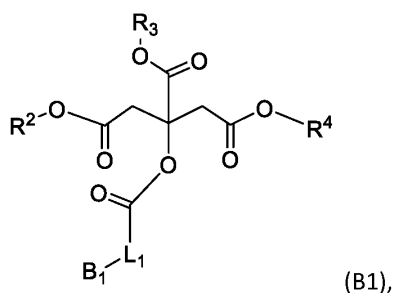
No.	Structure
89	

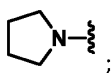
[0454] In embodiments, a compound of Formula (B) is selected from the group consisting of Compounds (65), (66), (67), (70), (71), (74), (75), (77), (81), (84), (87), (88), and (89).

[0455] In embodiments, a compound of Formula (B) is selected from the group consisting of Compounds (68), (69), (72), (73), (76), (78), (79), (80), (82), (83), (85), and (86).

- 5 **[0456]** In embodiments, a compound of Formula (B) excludes Compounds (68), (69), (72), (73), (76), (78), (79), (80), (82), (83), (85), and (86).

[0457] Also provided are compounds of Formula (B1):

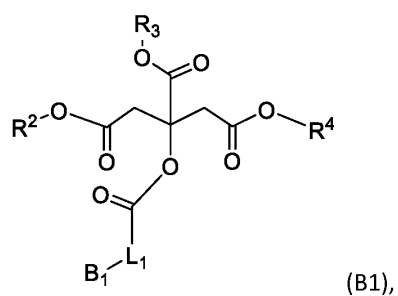


wherein B₁ is  ;

- 10 L₁ is as defined for Formula (B); and

R₂, R₃, and R₄ are straight-chain C₈ alkylene.

[0458] Also provided are compounds of Formula (B1):

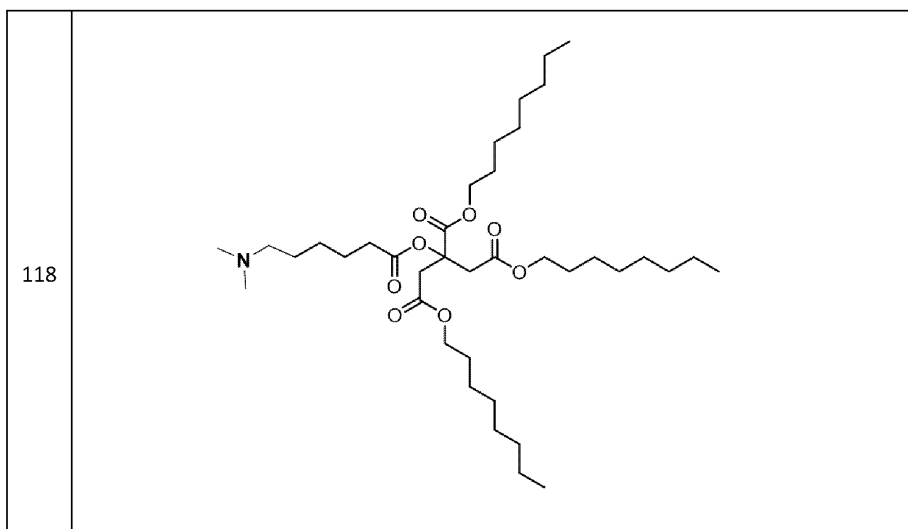


wherein B₁ is ;

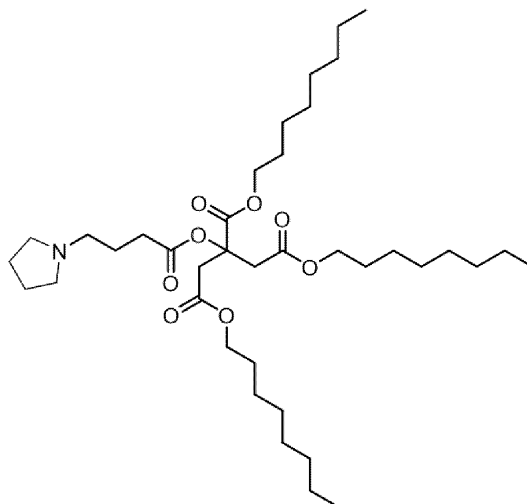
L₁ is straight-chain C₅ alkylene; and

R₂, R₃, and R₄ are as defined for Formula (B)

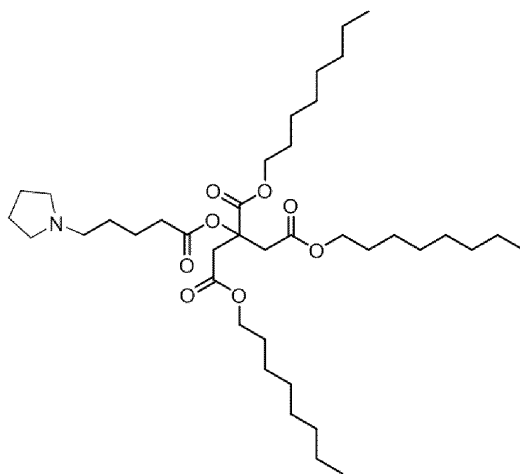
- 5 **[0459]** In embodiments, a compound of Formula (B) or (B1) is selected from the group consisting of Compounds (118)-(125), or a pharmaceutically acceptable salt thereof:

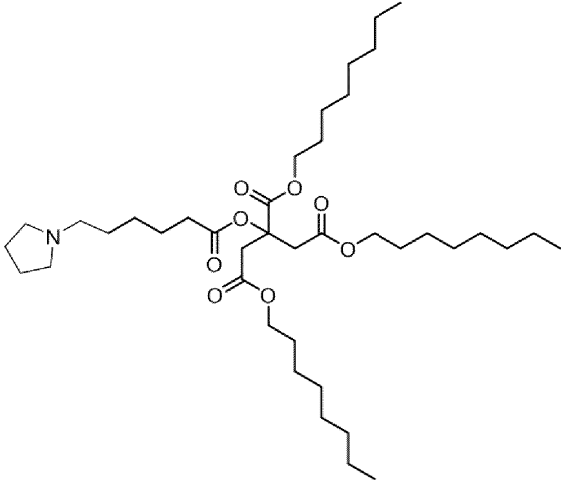
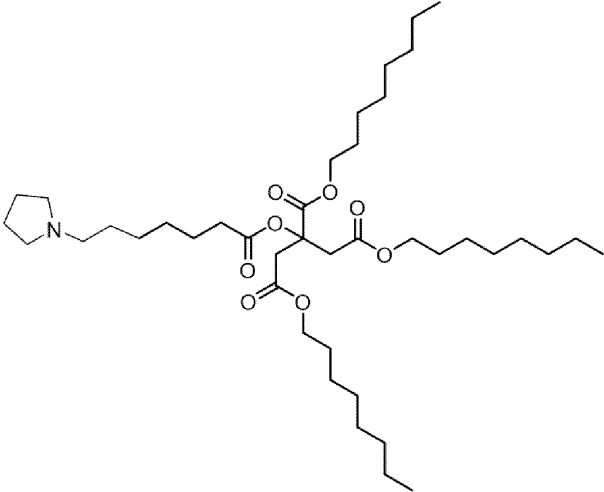
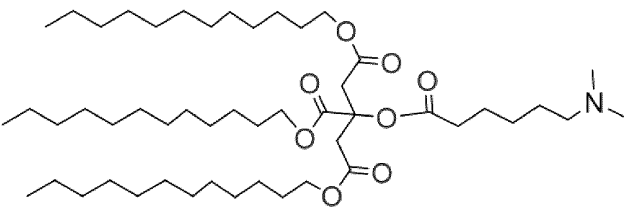


119



120



121	 <chem>CCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCC)C(=O)OCCCCCCCCN1CCCC1</chem>
122	 <chem>CCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCC)C(=O)OCCCCCCCCN1CCCC1</chem>
123	 <chem>CCCCCCCC(=O)OCC(=O)C(C(=O)OCCCCCCCC)C(=O)OCCCCCCCCN(C)C</chem>

124	
125	

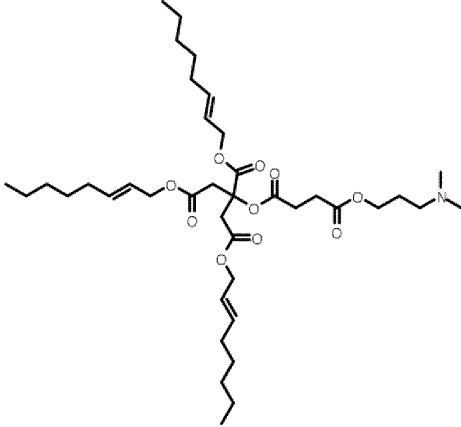
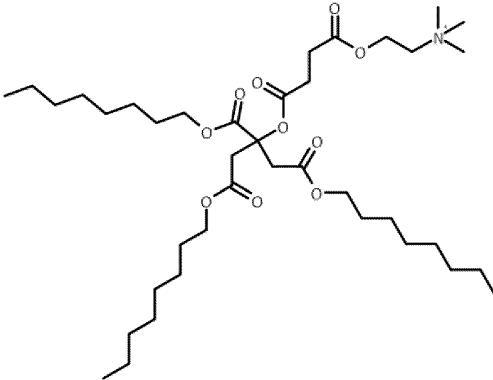
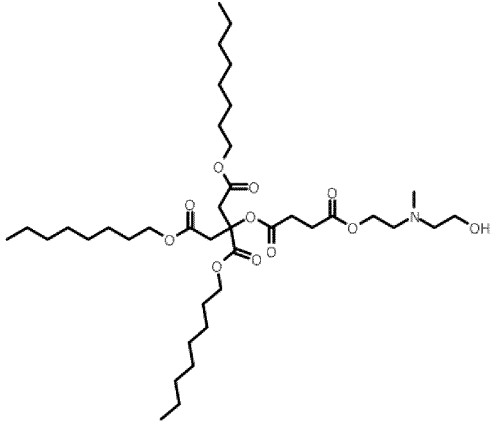
Exemplary Compounds

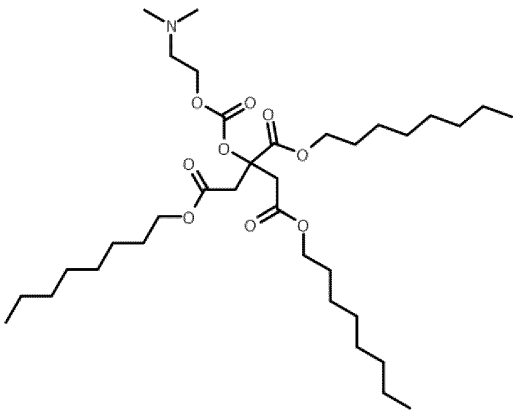
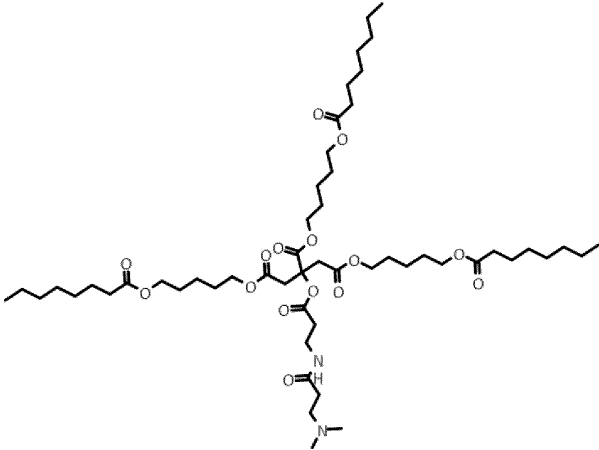
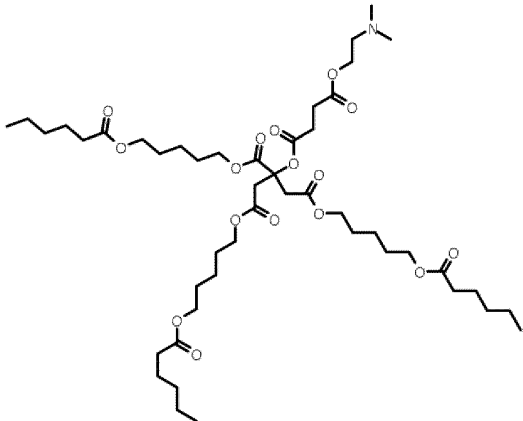
[0460] Exemplary compounds of the invention include those cationic lipids described in Table

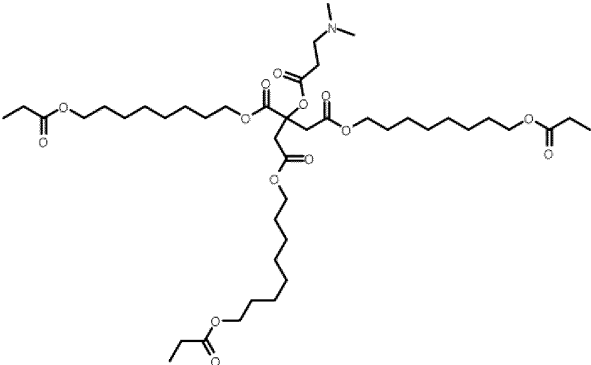
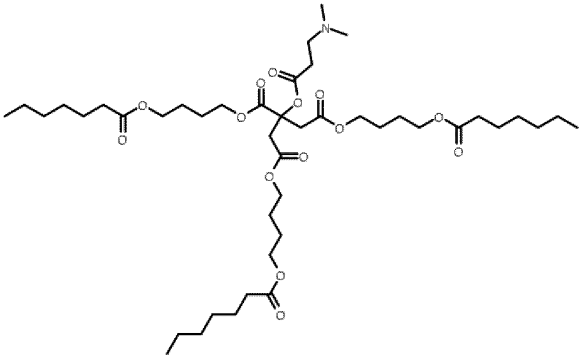
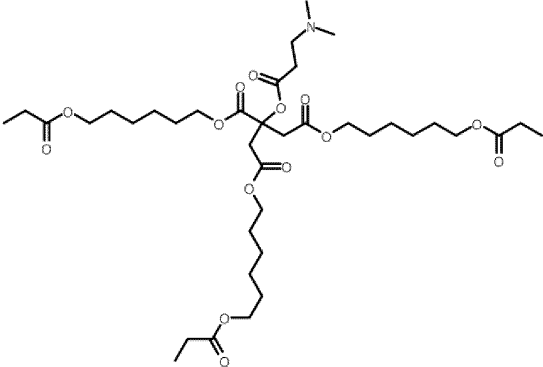
5 1.

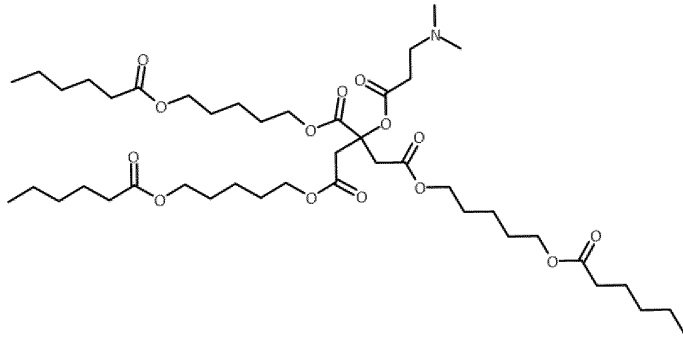
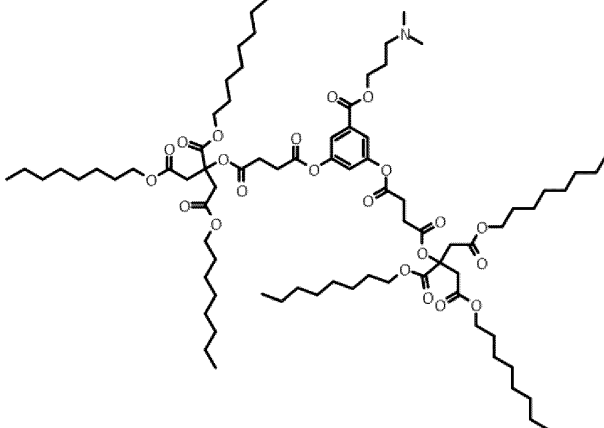
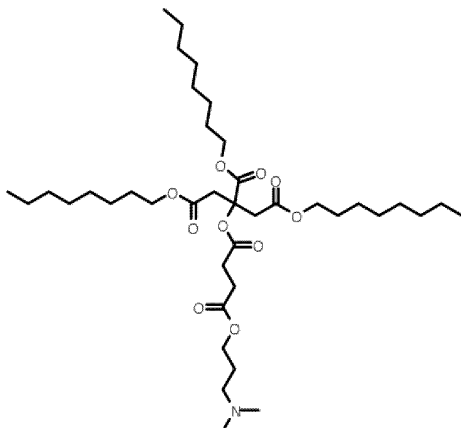
Table 1. Exemplary Cationic Lipids

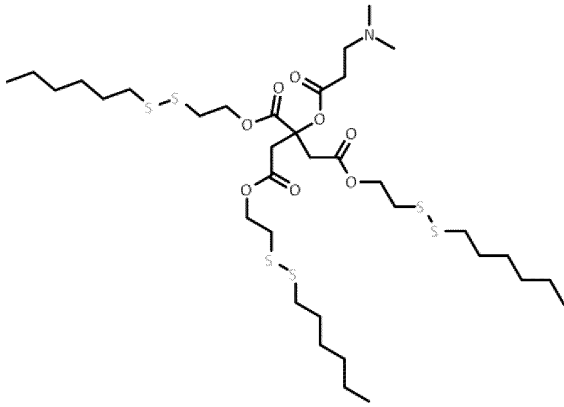
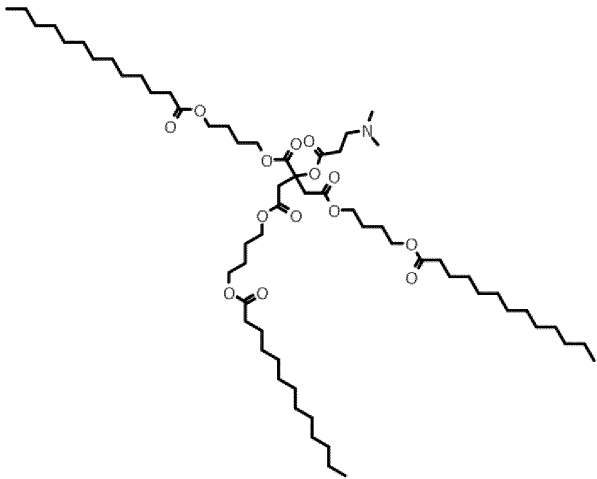
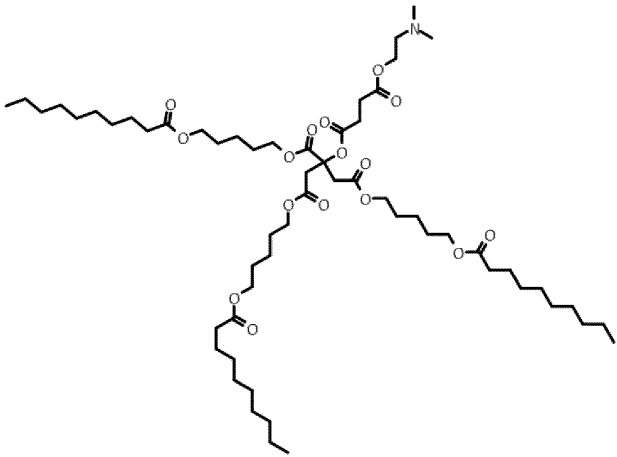
No.	Formula	Structure
1	(I)	

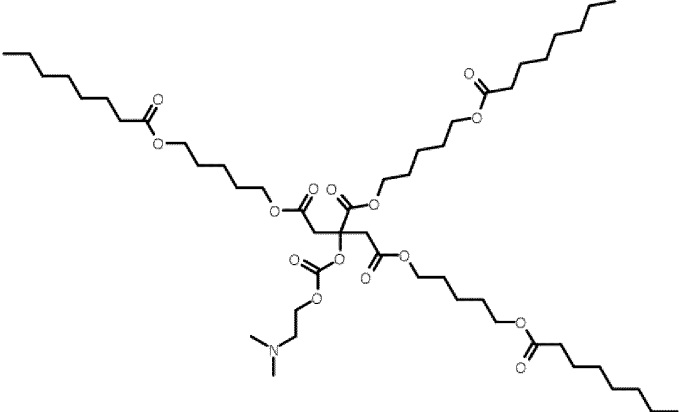
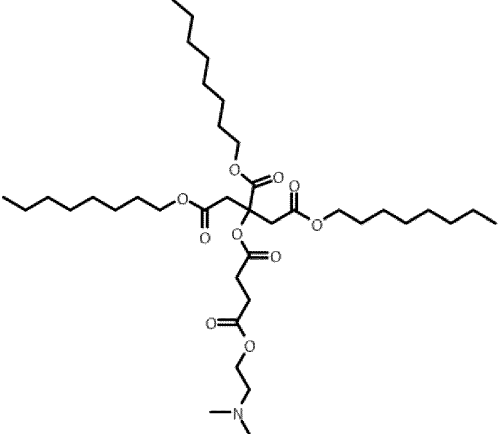
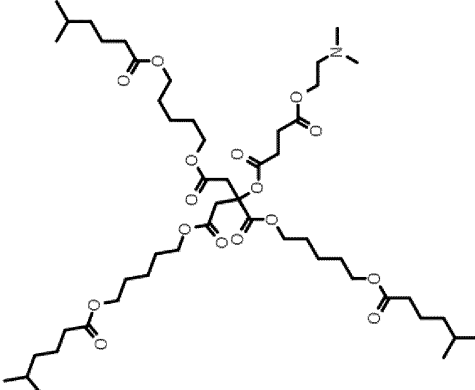
No.	Formula	Structure
2	(I)	 Chemical structure of compound 2, labeled (I). It is a central carbon atom bonded to four groups: a long alkyl chain (octyl) at the top, a long alkyl chain (octyl) at the bottom, a long alkyl chain (octyl) at the left, and a long alkyl chain (octyl) at the right. The central carbon is also bonded to two oxygen atoms, each connected to a carbonyl group (C=O). The carbonyl groups are further connected to a chain of three carbonyl groups (C=O), which is then connected to a dimethylamino group (N(CH3)2).
3	(I)	 Chemical structure of compound 3, labeled (I). It is a central carbon atom bonded to four groups: a long alkyl chain (octyl) at the top, a long alkyl chain (octyl) at the bottom, a long alkyl chain (octyl) at the left, and a long alkyl chain (octyl) at the right. The central carbon is also bonded to two oxygen atoms, each connected to a carbonyl group (C=O). The carbonyl groups are further connected to a chain of three carbonyl groups (C=O), which is then connected to a dimethylamino group (N(CH3)2).
4	(I)	 Chemical structure of compound 4, labeled (I). It is a central carbon atom bonded to four groups: a long alkyl chain (octyl) at the top, a long alkyl chain (octyl) at the bottom, a long alkyl chain (octyl) at the left, and a long alkyl chain (octyl) at the right. The central carbon is also bonded to two oxygen atoms, each connected to a carbonyl group (C=O). The carbonyl groups are further connected to a chain of three carbonyl groups (C=O), which is then connected to a dimethylamino group (N(CH3)2).

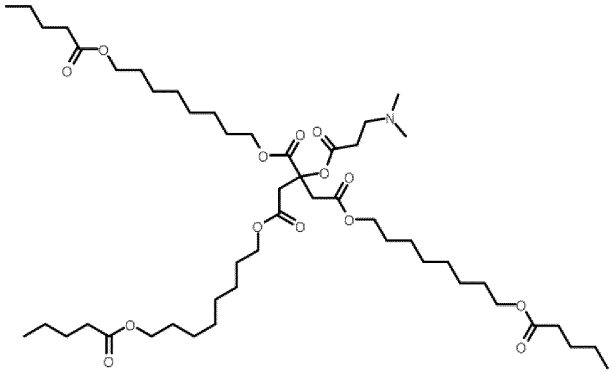
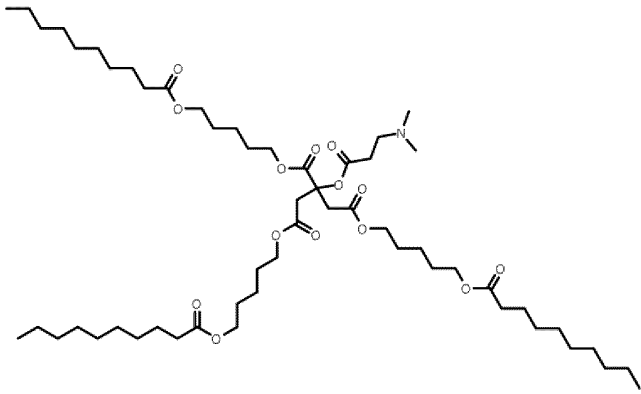
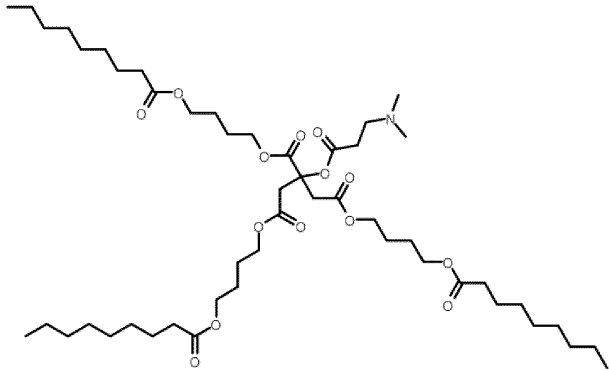
No.	Formula	Structure
6	(III)	
8	(IV)	
9	(I)	

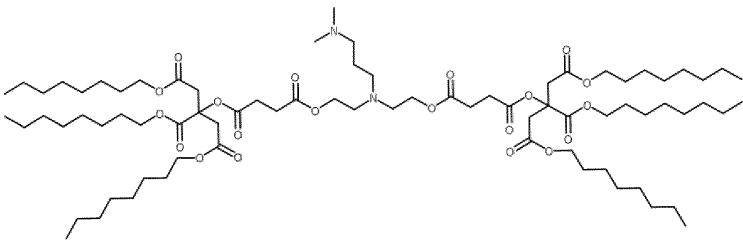
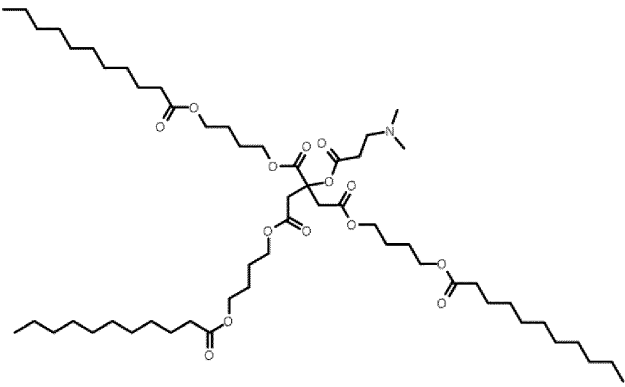
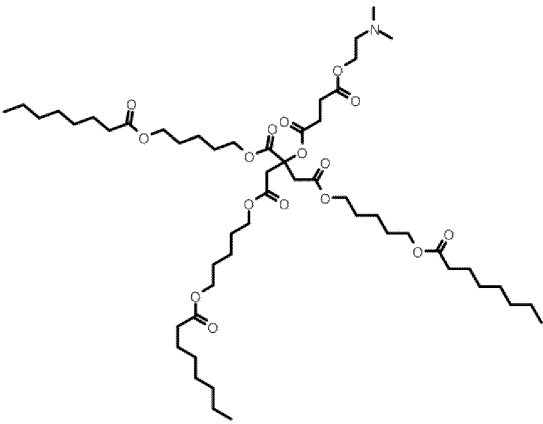
No.	Formula	Structure
11	(V)	
12	(V)	
13	(V)	

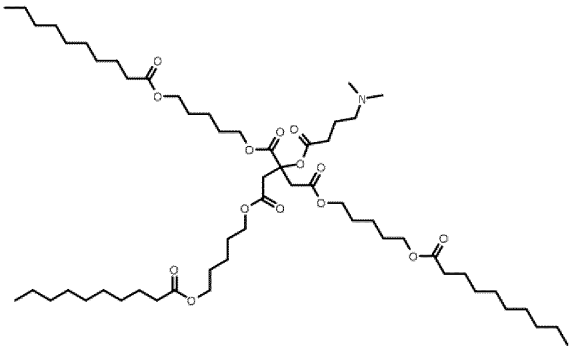
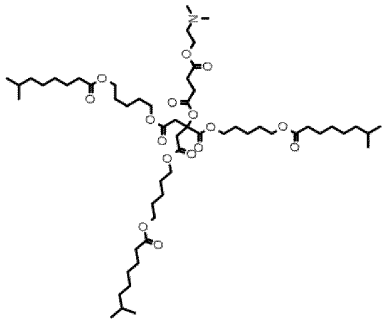
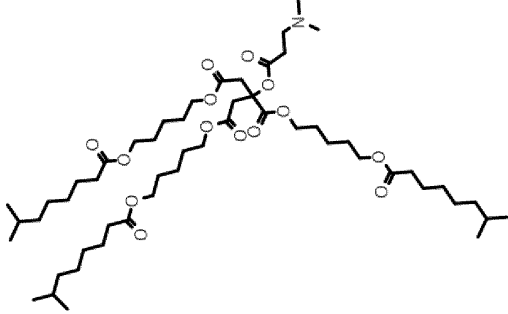
No.	Formula	Structure
14	(V)	 Chemical structure (V) is a complex molecule featuring a central carbon atom bonded to four long alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.
16	(VII)	 Chemical structure (VII) is a complex molecule featuring a central carbon atom bonded to four long alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.
17	(I)	 Chemical structure (I) is a complex molecule featuring a central carbon atom bonded to four long alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.

No.	Formula	Structure
21	(VI)	
22	(V)	
24	(I)	

No.	Formula	Structure
25	(V)	 Chemical structure of compound 25, a macrocyclic ester. The structure features a central macrocyclic ring with four long alkyl chains (octyl groups) attached via ester linkages. A dimethylamino group is also present on the ring.
26	(I)	 Chemical structure of compound 26, a macrocyclic ester. The structure features a central macrocyclic ring with four long alkyl chains (octyl groups) attached via ester linkages. A dimethylamino group is also present on the ring.
27	(I)	 Chemical structure of compound 27, a macrocyclic ester. The structure features a central macrocyclic ring with four long alkyl chains (octyl groups) attached via ester linkages. A dimethylamino group is also present on the ring.

No.	Formula	Structure
28	(V)	 Chemical structure 28 shows a central 1,3-dioxane ring. At position 4, there is a dimethylamino group (-N(CH₃)₂). At positions 2, 5, and 6, there are long-chain alkyl ester groups. The ester groups are: a 10-undecyloxy group at position 2, a 10-undecyloxy group at position 5, and a 10-undecyloxy group at position 6.
29	(V)	 Chemical structure 29 shows a central 1,3-dioxane ring. At position 4, there is a dimethylamino group (-N(CH₃)₂). At positions 2, 5, and 6, there are long-chain alkyl ester groups. The ester groups are: a 10-undecyloxy group at position 2, a 10-undecyloxy group at position 5, and a 10-undecyloxy group at position 6.
30	(V)	 Chemical structure 30 shows a central 1,3-dioxane ring. At position 4, there is a dimethylamino group (-N(CH₃)₂). At positions 2, 5, and 6, there are long-chain alkyl ester groups. The ester groups are: a 10-undecyloxy group at position 2, a 10-undecyloxy group at position 5, and a 10-undecyloxy group at position 6.

No.	Formula	Structure
31	(VII)	
32	(V)	
33	(I)	

No.	Formula	Structure
34	(V)	
35	(I)	
36	(V)	

No.	Formula	Structure
38	(V)	
39	(IV)	
40	(V)	

- [0461] In embodiments, a cationic lipid is Compound (1) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (2) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (3) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (4) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (6) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (8) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (9) or a pharmaceutically acceptable salt thereof.

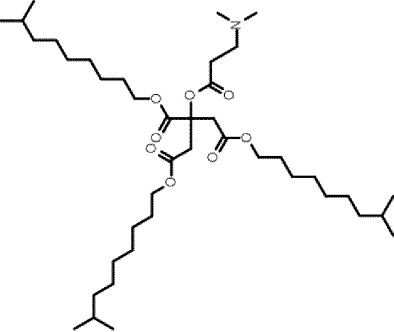
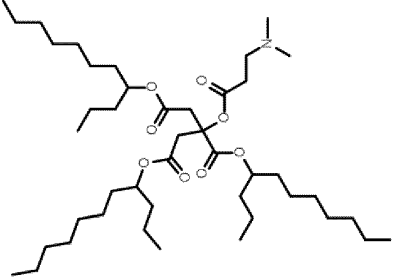
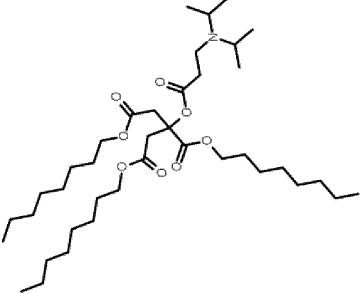
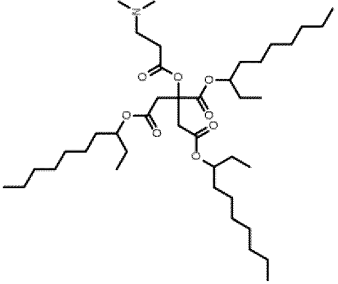
[0462] In embodiments, a cationic lipid is Compound (11) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (12) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (13) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (14) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (16) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (17) or a pharmaceutically acceptable salt thereof.

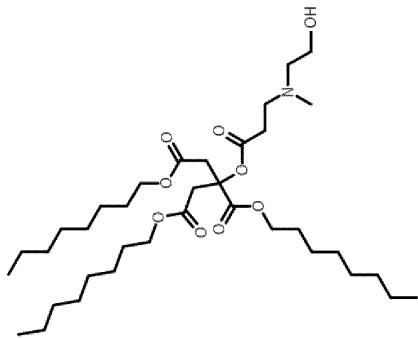
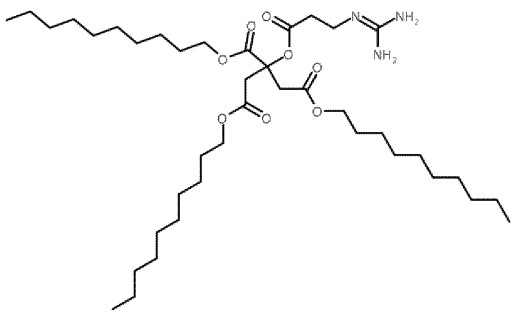
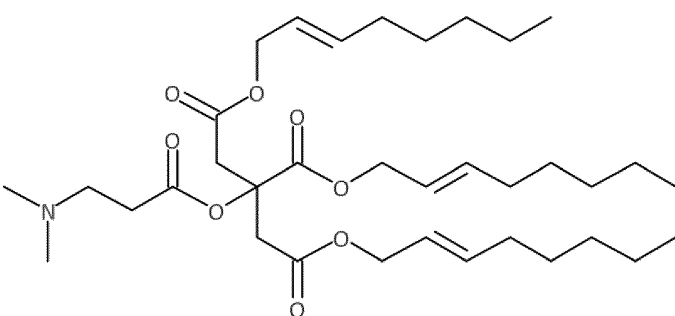
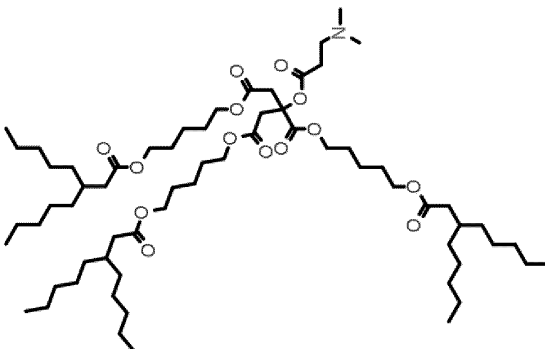
[0463] In embodiments, a cationic lipid is Compound (21) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (22) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (24) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (25) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (26) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (27) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (28) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (29) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (30) or a pharmaceutically acceptable salt thereof.

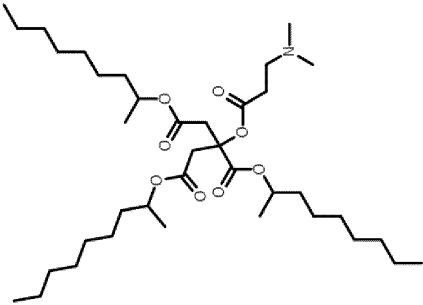
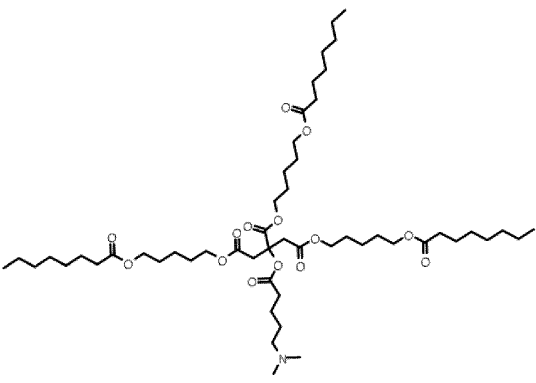
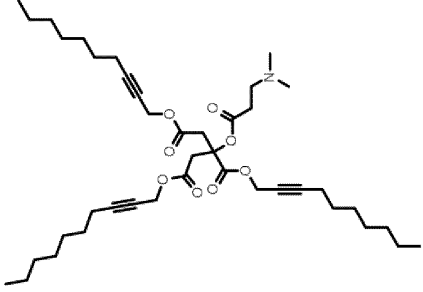
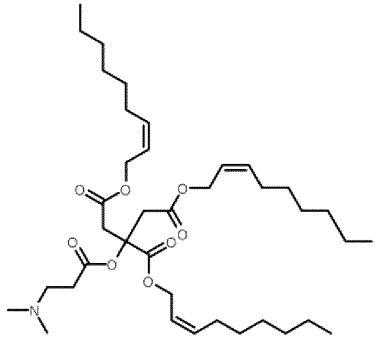
[0464] In embodiments, a cationic lipid is Compound (31) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (32) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (33) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (34) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (35) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (36) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (38) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (39) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (40) or a pharmaceutically acceptable salt thereof.

[0465] Exemplary compounds of the invention include those cationic lipids described in **Table 2**.

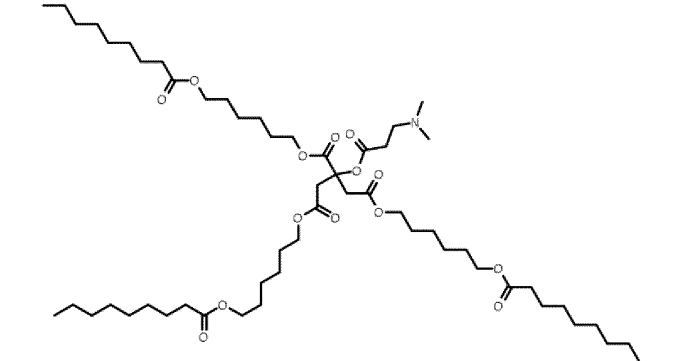
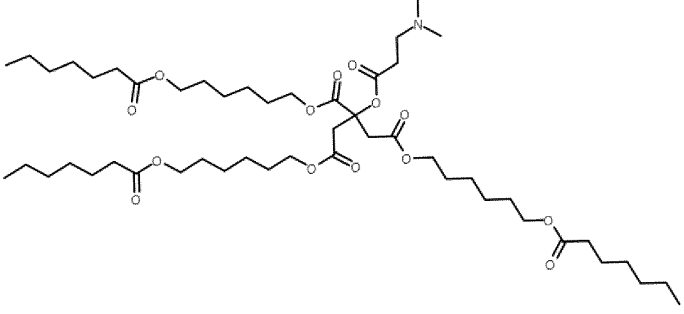
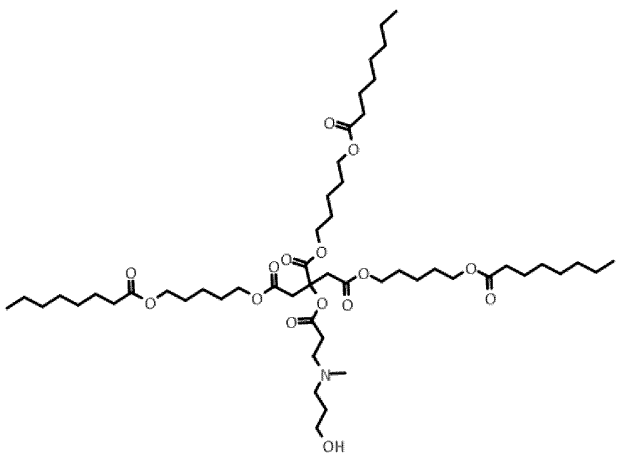
Table 2. Exemplary Cationic Lipids

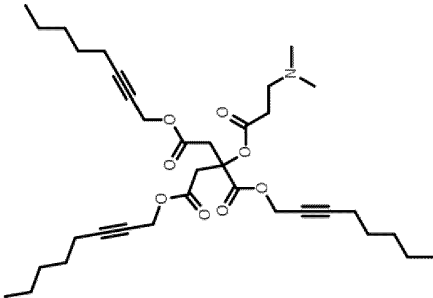
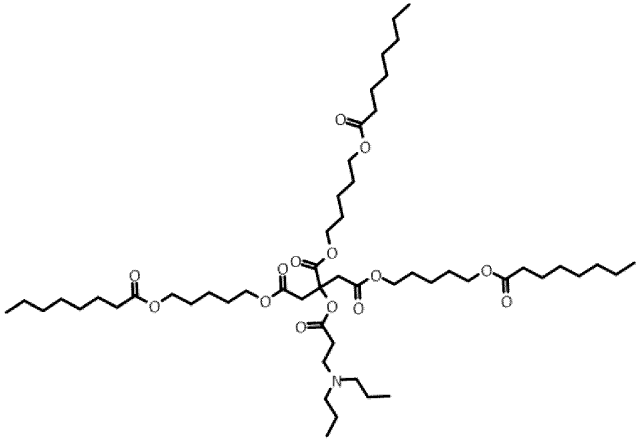
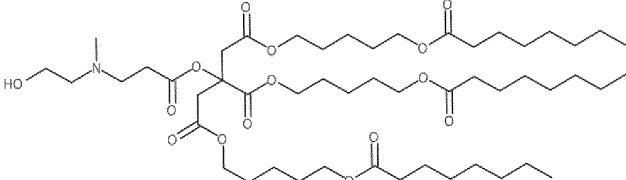
No.	Structure
41	 Chemical structure of cationic lipid 41. It features a central phosphorus atom bonded to four fatty acid chains. The chains are: a 16:0 chain (top-left), a 16:1 chain (top-right), a 16:0 chain (bottom-left), and a 16:1 chain (bottom-right). The phosphorus atom is also bonded to a trimethylammonium group (N⁺(CH₃)₃).
42	 Chemical structure of cationic lipid 42. It features a central phosphorus atom bonded to four fatty acid chains. The chains are: a 16:0 chain (top-left), a 16:1 chain (top-right), a 16:0 chain (bottom-left), and a 16:1 chain (bottom-right). The phosphorus atom is also bonded to a trimethylammonium group (N⁺(CH₃)₃).
43	 Chemical structure of cationic lipid 43. It features a central phosphorus atom bonded to four fatty acid chains. The chains are: a 16:0 chain (top-left), a 16:1 chain (top-right), a 16:0 chain (bottom-left), and a 16:1 chain (bottom-right). The phosphorus atom is also bonded to a trimethylammonium group (N⁺(CH₃)₃).
44	 Chemical structure of cationic lipid 44. It features a central phosphorus atom bonded to four fatty acid chains. The chains are: a 16:0 chain (top-left), a 16:1 chain (top-right), a 16:0 chain (bottom-left), and a 16:1 chain (bottom-right). The phosphorus atom is also bonded to a trimethylammonium group (N⁺(CH₃)₃).

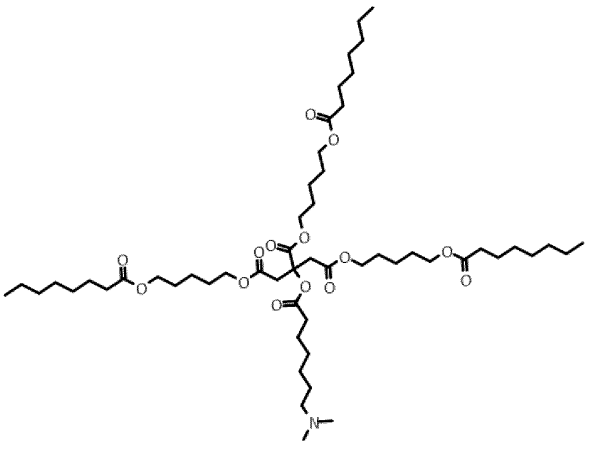
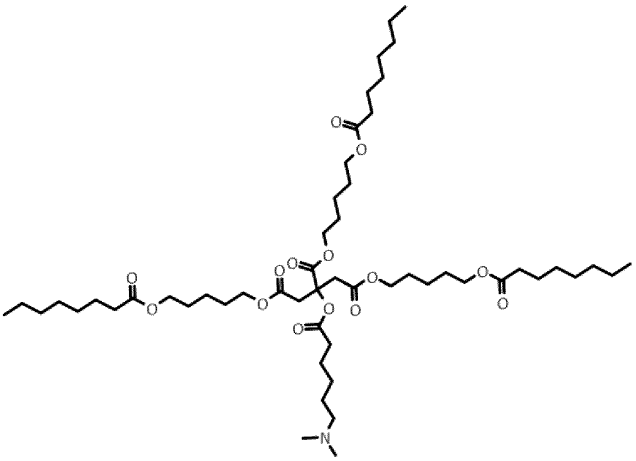
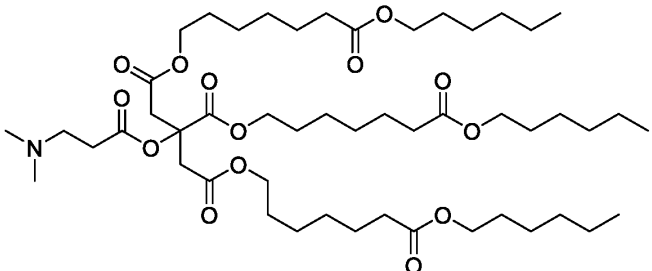
No.	Structure
45	
46	
47	
48	

No.	Structure
49	
50	
51	
52	

No.	Structure
53	
54	
55	
56	

No.	Structure
57	
58	
59	

No.	Structure
60	
61	
62	

No.	Structure
63	
64	
90	

[0466] In embodiments, a cationic lipid is Compound (41) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (42) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (43) or a pharmaceutically acceptable salt thereof.

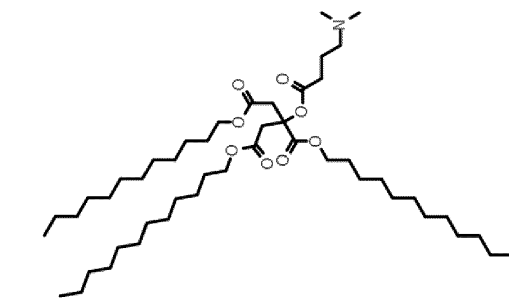
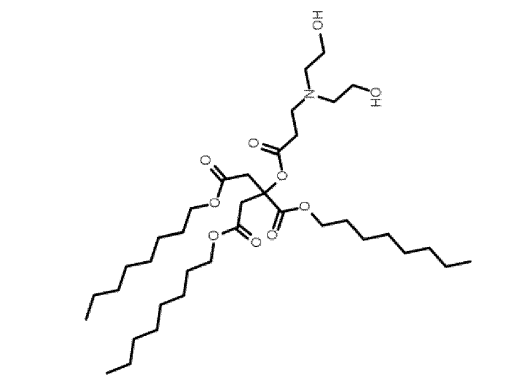
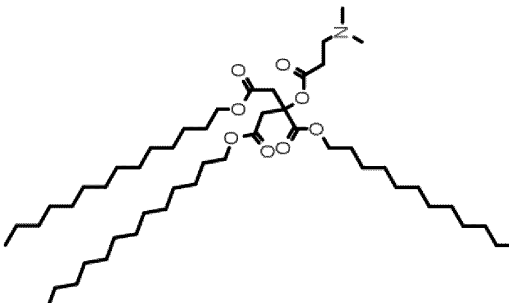
thereof. In embodiments, a cationic lipid is Compound (44) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (45) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (46) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (47) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (48) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (49) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (50) or a pharmaceutically acceptable salt thereof.

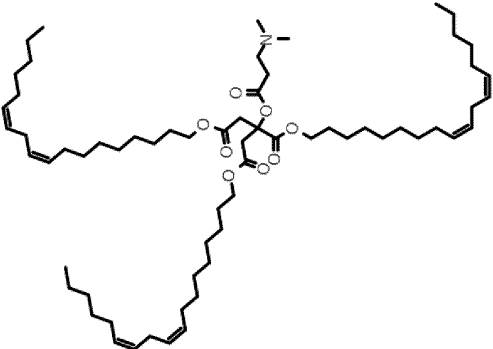
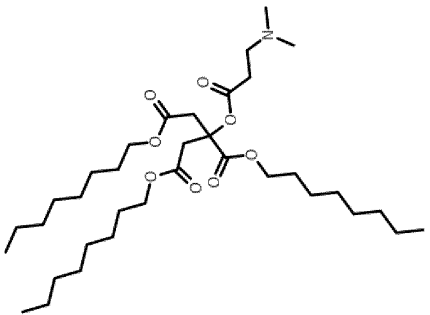
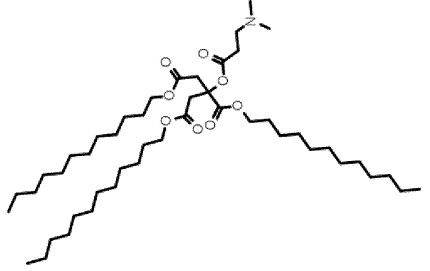
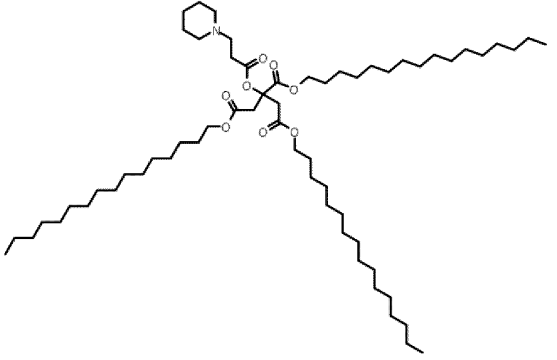
[0467] In embodiments, a cationic lipid is Compound (51) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (52) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (53) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (54) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (55) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (56) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (57) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (58) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (59) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (60) or a pharmaceutically acceptable salt thereof.

[0468] In embodiments, a cationic lipid is Compound (61) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (62) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (63) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (64) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (90) or a pharmaceutically acceptable salt thereof.

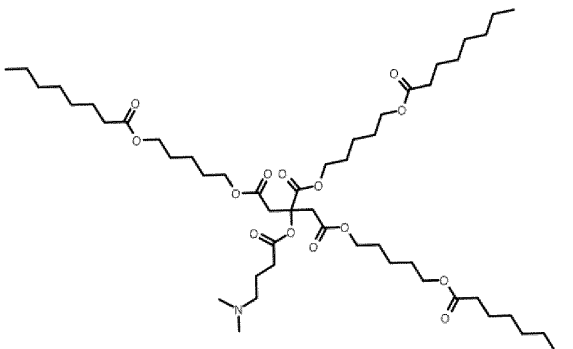
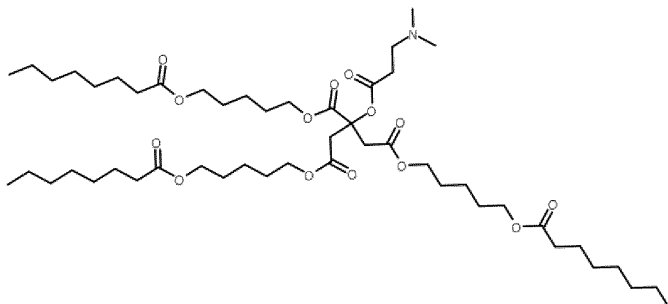
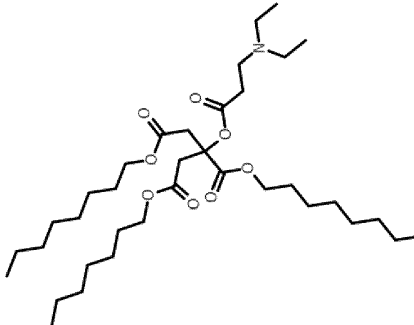
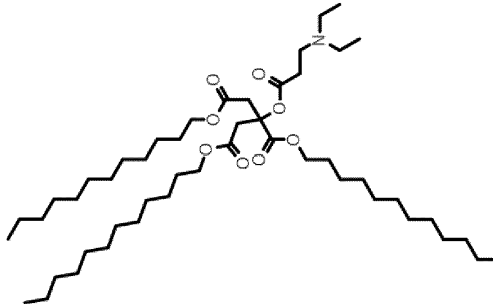
[0469] Exemplary compounds of the invention include those cationic lipids described in **Table 3**.

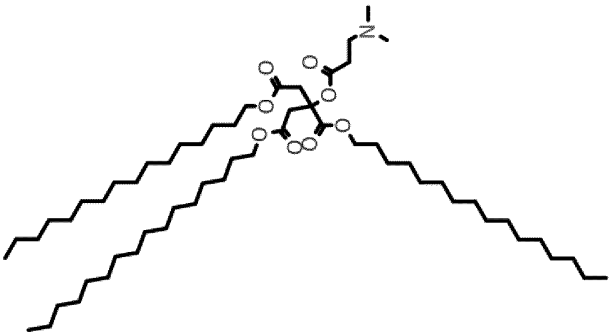
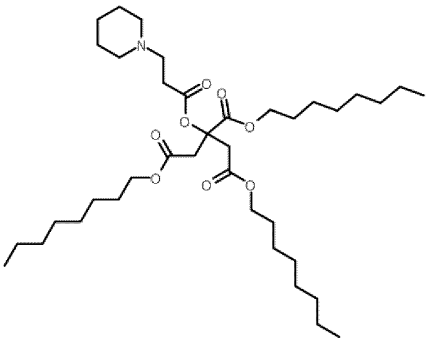
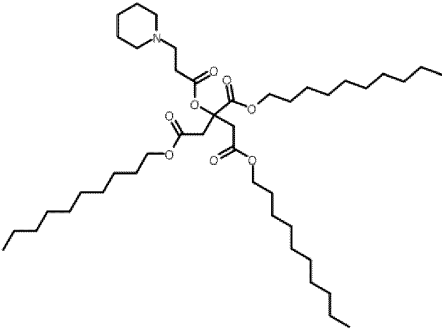
Table 3. Exemplary Cationic Lipids

No.	Structure
65	
66	
67	

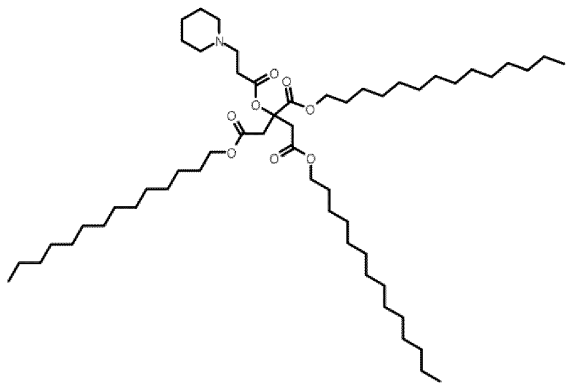
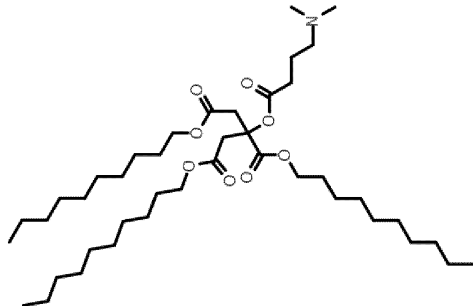
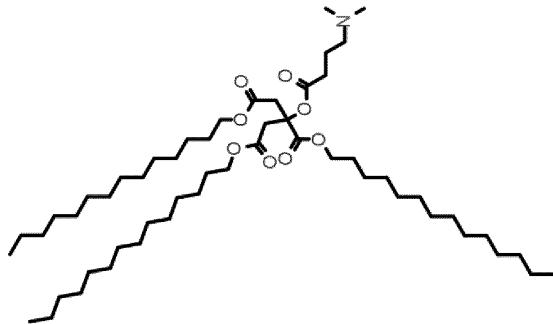
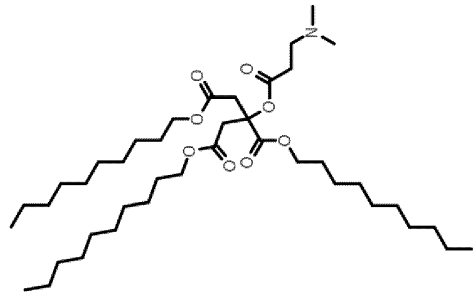
No.	Structure
68	 Chemical structure 68 is a complex molecule featuring a central core with three long, branched alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.
69	 Chemical structure 69 is a complex molecule featuring a central core with three long, branched alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.
70	 Chemical structure 70 is a complex molecule featuring a central core with three long, branched alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.
71	 Chemical structure 71 is a complex molecule featuring a central core with three long, branched alkyl chains and a dimethylamino group. The structure is highly branched and includes several ester and ether linkages.

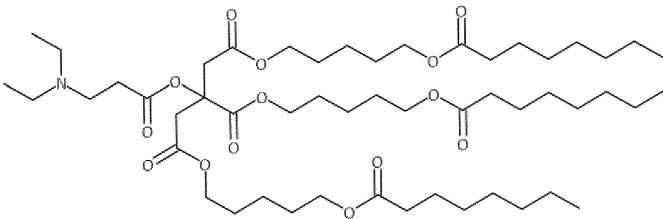
No.	Structure
72	
73	
74	

No.	Structure
75	
76	
77	
78	

No.	Structure
79	
80	
81	

No.	Structure
82	
83	
84	

No.	Structure
85	
86	
87	
88	

No.	Structure
89	

[0470] In embodiments, a cationic lipid is Compound (65) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (66) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (67) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (68) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (69) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (70) or a pharmaceutically acceptable salt thereof.

[0471] In embodiments, a cationic lipid is Compound (71) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (72) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (73) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (74) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (75) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (76) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (77) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (78) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (79) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (80) or a pharmaceutically acceptable salt thereof.

[0472] In embodiments, a cationic lipid is Compound (81) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (82) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (83) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (84) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (85) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (86) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (87) or a pharmaceutically acceptable salt thereof.

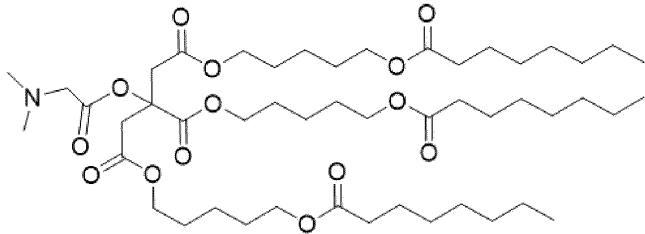
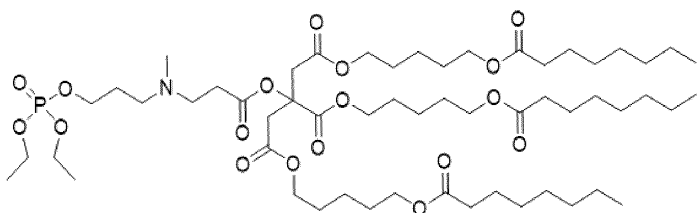
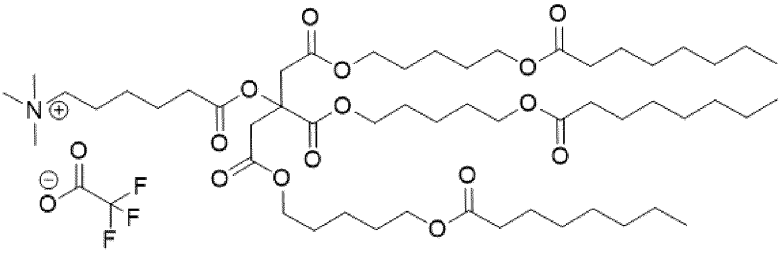
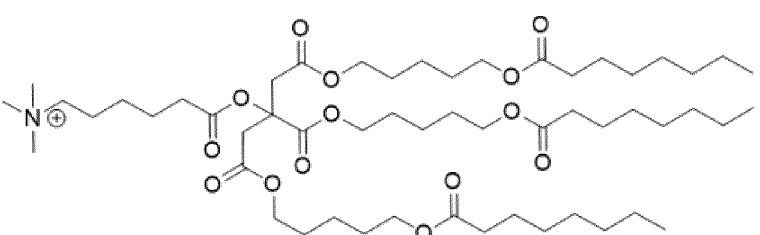
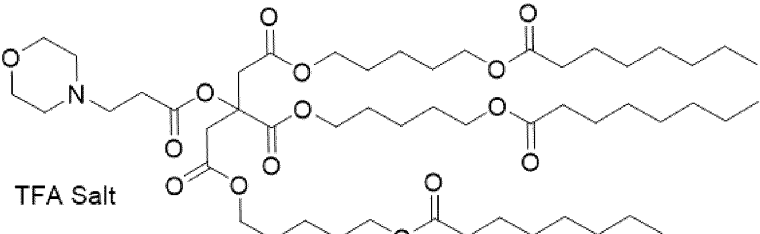
thereof. In embodiments, a cationic lipid is Compound (88) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (89) or a pharmaceutically acceptable salt thereof.

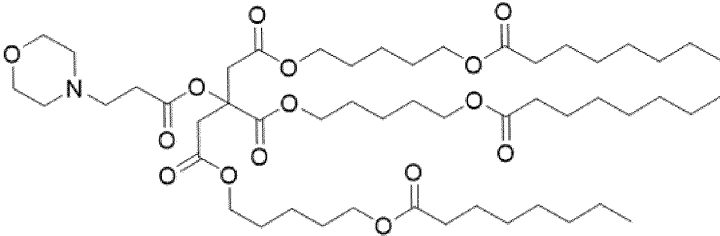
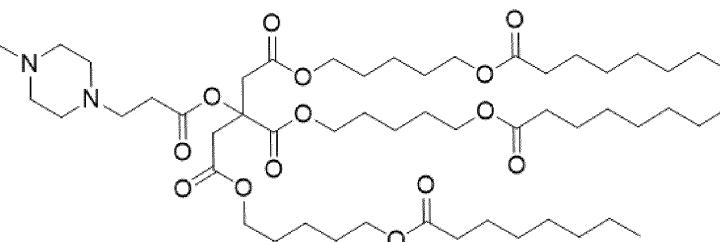
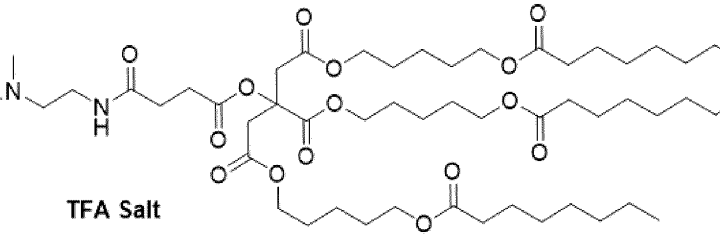
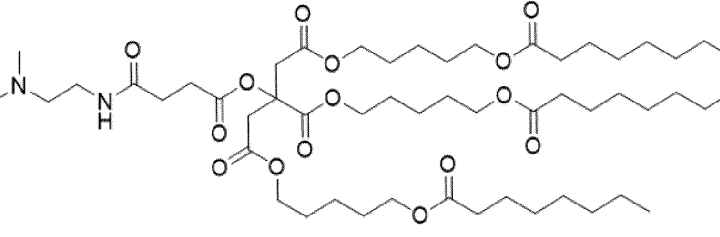
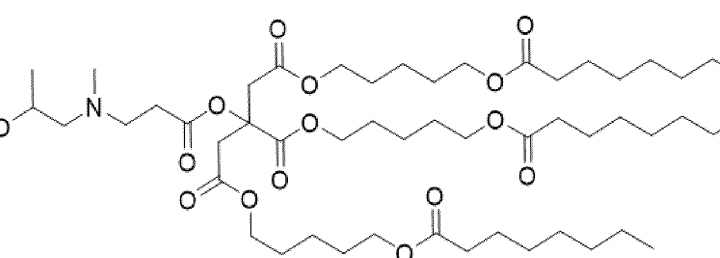
[0473] Exemplary compounds of the invention include those cationic lipids described in Table

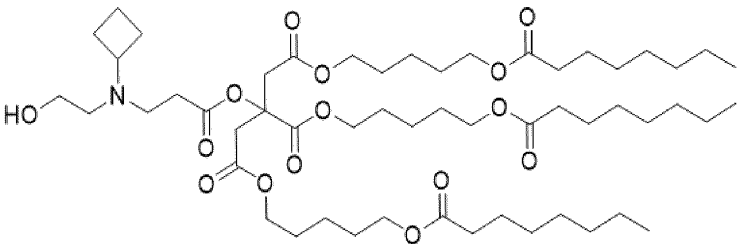
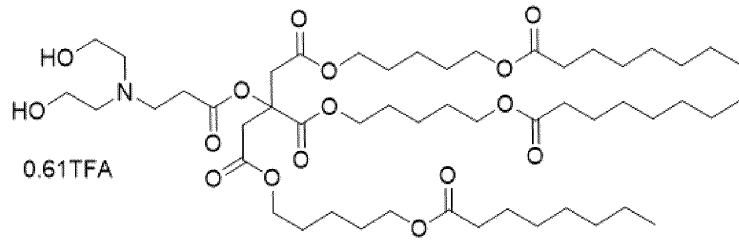
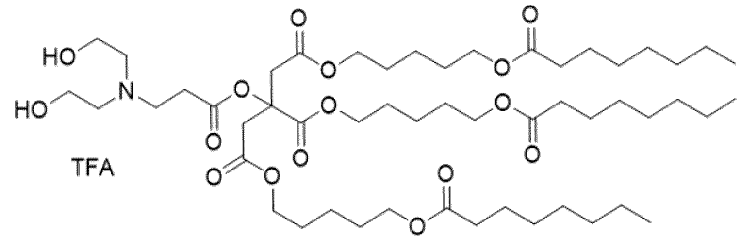
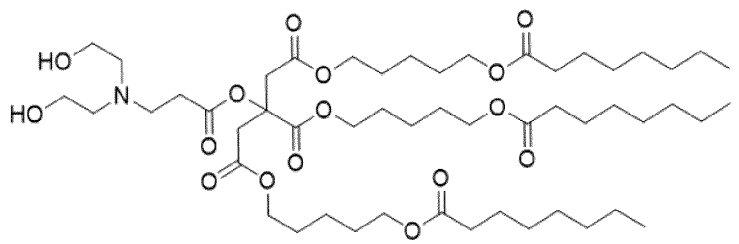
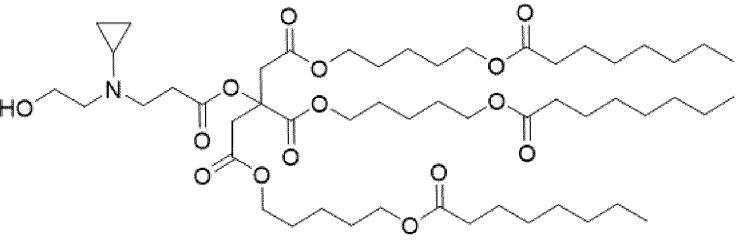
5 9.

Table 9. Exemplary Cationic Lipids

No.	Structure
91	Chemical structure of Compound 91: A cationic lipid. It features a central carbon atom bonded to a trimethylammonium group (N⁺(CH₃)₃) and four long-chain fatty acid tails. The tails are connected via ester linkages to a central carbon atom. The tails are: one octyl chain, one decyl chain, one dodecyl chain, and one hexadecyl chain.
92	Chemical structure of Compound 92: A cationic lipid. It features a central carbon atom bonded to a trimethylammonium group (N⁺(CH₃)₃) and four long-chain fatty acid tails. The tails are connected via ester linkages to a central carbon atom. The tails are: one octyl chain, one decyl chain, one dodecyl chain, and one hexadecyl chain.
93	Chemical structure of Compound 93: A cationic lipid. It features a central carbon atom bonded to a trimethylammonium group (N⁺(CH₃)₃) and four long-chain fatty acid tails. The tails are connected via ester linkages to a central carbon atom. The tails are: one octyl chain, one decyl chain, one dodecyl chain, and one hexadecyl chain.

No.	Structure
94	
95	
96	
97	
98	 TFA Salt

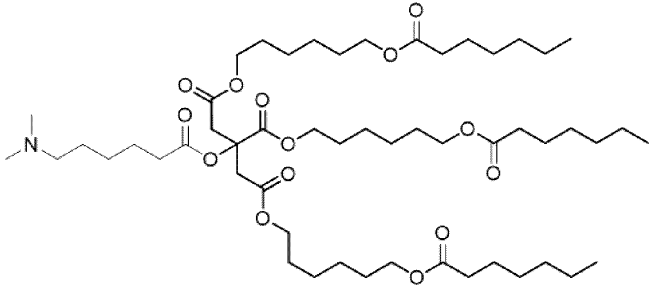
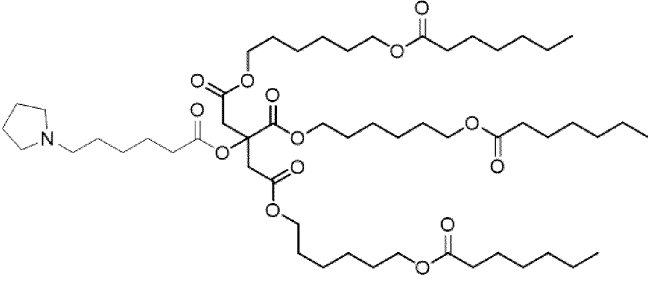
No.	Structure
99	
100	
101	 TFA Salt
102	
103	

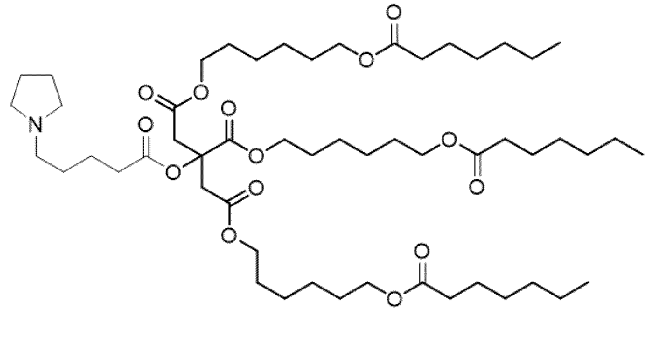
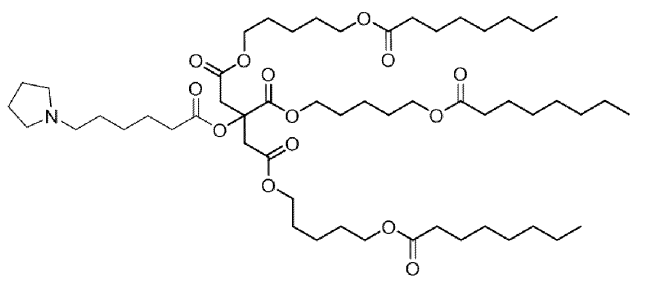
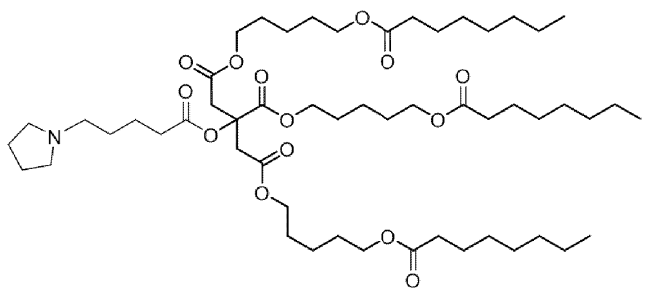
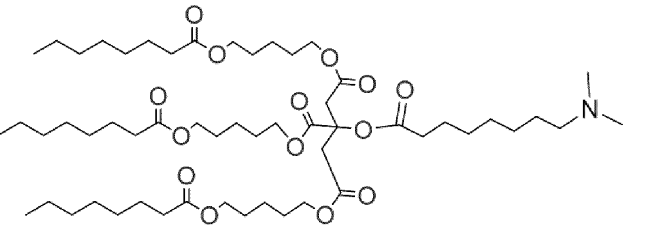
No.	Structure
104	
105	 0.61TFA
106	 TFA
107	
108	

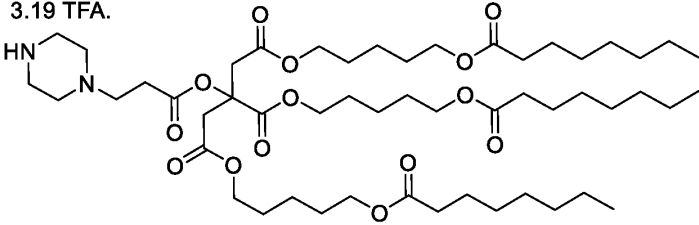
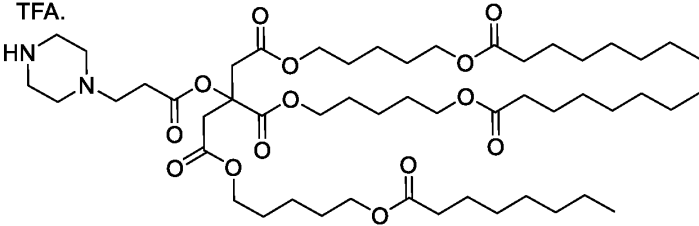
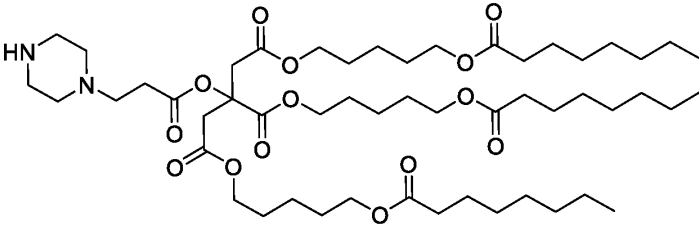
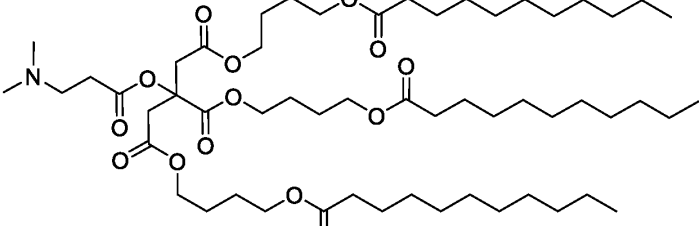
thereof. In embodiments, a cationic lipid is Compound (101) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (102) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (103) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (104) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (105) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (106) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (107) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (108) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (109) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (110) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (111) or a pharmaceutically acceptable salt thereof.

[0475] Exemplary compounds of the invention include those cationic lipids described in **Table 10**.

Table 10. Exemplary Cationic Lipids

No.	Structure
112	
113	

No.	Structure
114	
115	
116	
117	

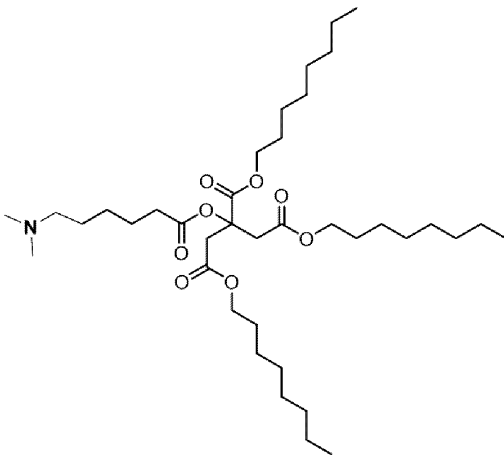
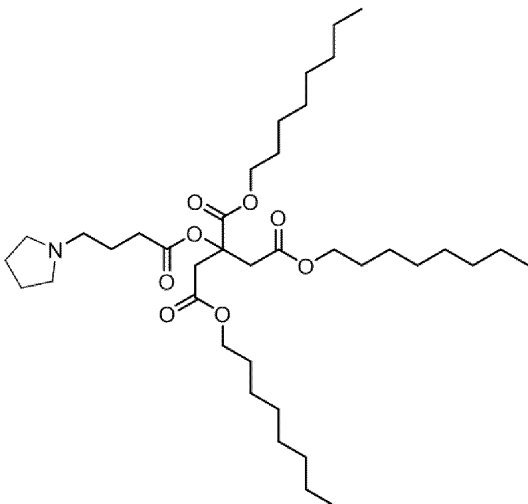
No.	Structure
126	3.19 TFA. 
127	TFA. 
128	
129	

[0476] In embodiments, a cationic lipid is Compound (112) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (113) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (114) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (115) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (116) or a pharmaceutically acceptable salt thereof.

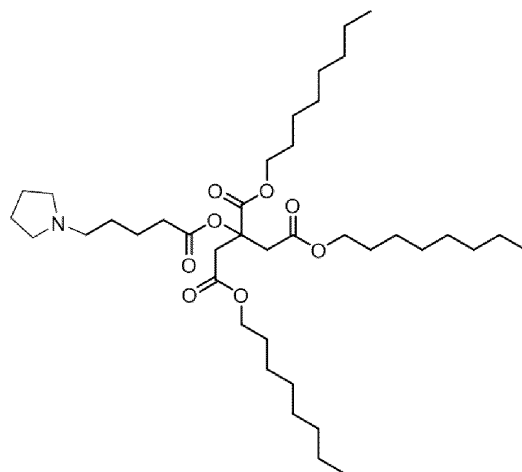
- salt thereof. In embodiments, a cationic lipid is Compound (117) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (126) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (127) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (128) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (129) or a pharmaceutically acceptable salt thereof.

[0477] Exemplary compounds of the invention include those cationic lipids described in **Table 11**.

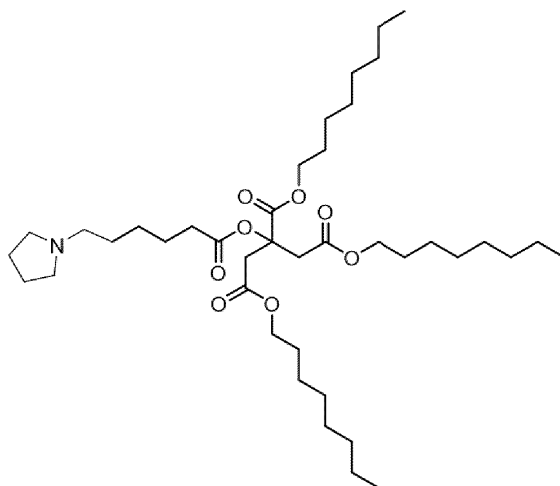
Table 11. Exemplary Cationic Lipids

118	
119	

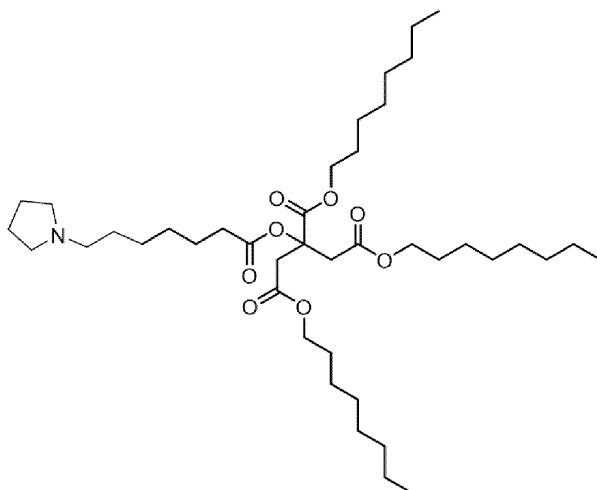
120

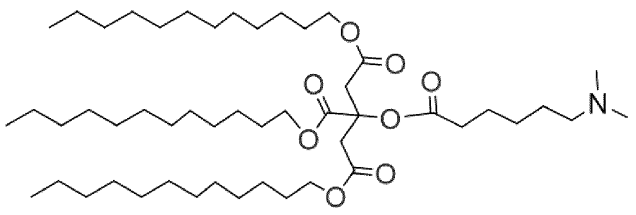
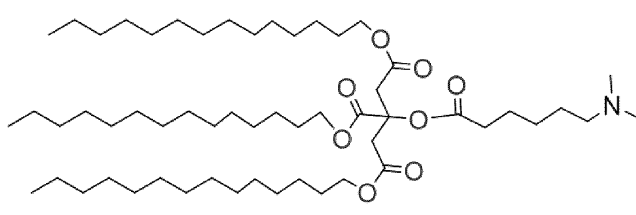
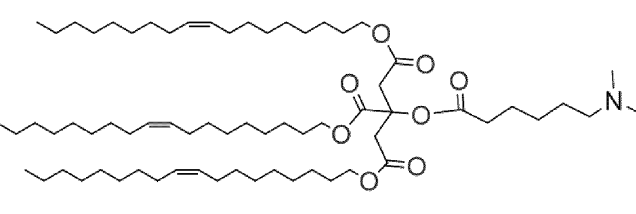


121



122



123	
124	
125	

[0478] In embodiments, a cationic lipid is Compound (118) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (119) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (120) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (121) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (122) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (123) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (124) or a pharmaceutically acceptable salt thereof. In embodiments, a cationic lipid is Compound (125) or a pharmaceutically acceptable salt thereof.

[0479] As further described herein, compositions comprising any compound of the invention include compositions comprising the cationic lipid of any one of the preceding embodiments, one or more non-cationic lipids, one or more cholesterol-based lipids, and/or one or more PEG-modified lipids. In embodiments, this composition is a lipid nanoparticle. In embodiments, the one or more cationic lipid(s) constitute(s) about 30 mol %-60 mol % of the lipid nanoparticle. In embodiments, the one or more non-cationic lipid(s) constitute(s) 10 mol%-50 mol% of the lipid

nanoparticle. In embodiments, the one or more PEG-modified lipid(s) constitute(s) 1 mol%-10 mol% of the lipid nanoparticle. In embodiments, the cholesterol-based lipid constitutes 10 mol%-50 mol% of the lipid nanoparticle. In embodiments, the lipid nanoparticle encapsulates a nucleic acid, optionally an mRNA encoding a peptide or protein. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 50%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 55%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 60%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 65%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 70%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 75%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 80%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 85%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 90%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 95%.

[0480] In embodiments, the composition of any one of the preceeding embodiments is for use in therapy.

[0481] In embodiments, the composition of any one of the preceeding embodiments is for use in a method of treating or preventing a disease amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.

[0482] In embodiments, the composition is administered intravenously, intrathecally or intramuscularly, or by pulmonary delivery, optionally through nebulization or use of an inhaler (e.g., a metered dose inhaler, a dry powder inhaler, or a soft mist inhaler).

[0483] Mucosal routes of administration such as oral and intranasal administration can elicit immune responses at both the local and distal mucosal sites. Such routes of administration can also give rise to systemic immune responses. As such, there is an interest in utilising routes of administration such as intranasal when administering the compositions of the present invention particularly with a view to generating protective immunity. In embodiments, the methods and compositions of the invention may induce mucosal immunity in a subject. In embodiments the

methods and compositions of the invention may induce mucosal immunity at distal mucosal sites. In embodiments, the methods and compositions of the invention may generate protective mucosal immunity. By “protective mucosal immunity” is meant immunity or an immune response against an infectious agent which is exhibited by a subject, whereby said immunity or immune response prevents or ameliorates an infection or reduces one or more symptoms thereof. The compositions and methods described herein may elicit an antibody response in a subject. In some embodiments said antibody may be IgA. In some embodiments, said antibody may be IgG.

[0484] During an infection or following a vaccination during which time a subject is exposed to a particular antigen, antigen specific T cells are activated, proliferate and then differentiate into effector cells. These effector cells serve to help clear the infection but most of these cells then go on to die. The remaining memory cells can afford protection and/or an increased response in the event of the subject encountering that same antigen again at a later time. Accordingly, it would be beneficial to identify methods and compositions that are capable of eliciting an antigen-specific T cell response, for example by inducing a CD4 memory T cell population. The compositions and methods described herein may enable a subject to produce an CD4 memory T cell population. In some embodiments, the compositions and methods described herein may induce a CD4 memory T cell population in a subject, optionally wherein said population is induced in the mucosa.

20 **Synthesis of Compounds**

[0485] The compounds of the invention as described herein can be prepared according to methods known in the art, including as described herein.

Nucleic Acids

25 [0486] The compounds of the invention as described herein can be used to prepare compositions useful for the delivery of nucleic acids.

Synthesis of Nucleic Acids

[0487] Nucleic acids according to the present invention may be synthesized according to any known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA

template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (*e.g.*, T3, T7, mutated T7 or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor. The exact conditions will vary according to the specific application.

- 5 **[0488]** In some embodiments, for the preparation of mRNA according to the invention, a DNA template is transcribed *in vitro*. A suitable DNA template typically has a promoter, for example a T3, T7, mutated T7 or SP6 promoter, for *in vitro* transcription, followed by desired nucleotide sequence for desired mRNA and a termination signal.

- 10 **[0489]** Desired mRNA sequence(s) according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence (*e.g.*, an enzyme sequence), a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

Modified mRNA

- 20 **[0490]** In some embodiments, mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Modified mRNA includes nucleotide modifications in the RNA. A modified mRNA according to the invention can thus include nucleotide modification that are, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as *e.g.*, 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-

carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queuosine, beta-D-mannosyl-queuosine, wybutosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art *e.g.*, from the U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642, the disclosures of which are incorporated by reference in their entirety.

Pharmaceutical Formulations of Cationic Lipids and Nucleic Acids

[0491] In certain embodiments, the compounds of the invention as described herein, as well as pharmaceutical and liposomal compositions comprising such lipids, can be used in formulations to facilitate the delivery of encapsulated materials (*e.g.*, one or more polynucleotides such as mRNA) to, and subsequent transfection of one or more target cells. For example, in certain embodiments cationic lipids described herein (and compositions such as liposomal compositions comprising such lipids) are characterized as resulting in one or more of receptor-mediated endocytosis, clathrin-mediated and caveolae-mediated endocytosis, phagocytosis and 187ncapsule187ytosis, fusogenicity, endosomal or lysosomal disruption and/or releasable properties that afford such compounds advantages relative other similarly classified lipids.

[0492] According to the present invention, a nucleic acid, *e.g.*, mRNA encoding a protein (*e.g.*, a full length, fragment or portion of a protein) as described herein may be delivered via a delivery vehicle comprising a compound of the invention as described herein.

[0493] As used herein, the terms "delivery vehicle," "transfer vehicle," "nanoparticle," or grammatical equivalents thereof, are used interchangeably.

[0494] For example, the present invention provides a composition (*e.g.*, a pharmaceutical composition) comprising a compound described herein and one or more polynucleotides. A composition (*e.g.*, a pharmaceutical composition) may further comprise one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and/or one or more PEG-modified lipids.

- [0495] In certain embodiments a composition exhibits an enhanced (*e.g.*, increased) ability to transfect one or more target cells. Accordingly, also provided herein are methods of transfecting one or more target cells. Such methods generally comprise the step of contacting the one or more target cells with the cationic lipids and/or pharmaceutical compositions disclosed herein (*e.g.*, a liposomal formulation comprising a compound described herein encapsulating one or more polynucleotides) such that the one or more target cells are transfected with the materials encapsulated therein (*e.g.*, one or more polynucleotides). As used herein, the terms “transfect” or “transfection” refer to the intracellular introduction of one or more encapsulated materials (*e.g.*, nucleic acids and/or polynucleotides) into a cell (*e.g.*, into a target cell). The introduced polynucleotide may be stably or transiently maintained in the target cell. The term “transfection efficiency” refers to the relative amount of such encapsulated material (*e.g.*, polynucleotides) taken up by, introduced into, and/or expressed by the target cell which is subject to transfection. In practice, transfection efficiency may be estimated by the amount of a reporter polynucleotide product produced by the target cells following transfection. In certain embodiments, the compounds and pharmaceutical compositions described herein demonstrate high transfection efficiencies thereby improving the likelihood that appropriate dosages of the encapsulated materials (*e.g.*, one or more polynucleotides) will be delivered to the site of pathology and subsequently expressed, while at the same time minimizing potential systemic adverse effects or toxicity associated with the compound or their encapsulated contents.
- [0496] Following transfection of one or more target cells by, for example, the polynucleotides encapsulated in the one or more lipid nanoparticles comprising the pharmaceutical or liposomal compositions disclosed herein, the production of the product (*e.g.*, a polypeptide or protein) encoded by such polynucleotide may be stimulated and the capability of such target cells to express the polynucleotide and produce, for example, a polypeptide or protein of interest is enhanced. For example, transfection of a target cell by one or more compounds or pharmaceutical compositions encapsulating mRNA will enhance (*i.e.*, increase) the production of the protein or enzyme encoded by such mRNA.
- [0497] Further, delivery vehicles described herein (*e.g.*, liposomal delivery vehicles) may be prepared to preferentially distribute to other target tissues, cells or organs, such as the heart, lungs, kidneys, spleen. In embodiments, the lipid nanoparticles of the present invention may be prepared to achieve enhanced delivery to the target cells and tissues. For example, polynucleotides (*e.g.*, mRNA) encapsulated in one or more of the compounds or pharmaceutical and liposomal compositions described herein can be delivered to and/or transfect targeted cells

or tissues. In some embodiments, the encapsulated polynucleotides (*e.g.*, mRNA) are capable of being expressed and functional polypeptide products produced (and in some instances excreted) by the target cell, thereby conferring a beneficial property to, for example the target cells or tissues. Such encapsulated polynucleotides (*e.g.*, mRNA) may encode, for example, a hormone, enzyme, receptor, polypeptide, peptide or other protein of interest.

Liposomal Delivery Vehicles

[0498] In some embodiments, a composition is a suitable delivery vehicle. In embodiments, a composition is a liposomal delivery vehicle, *e.g.*, a lipid nanoparticle.

[0499] The terms “liposomal delivery vehicle” and “liposomal composition” are used interchangeably.

[0500] Enriching liposomal compositions with one or more of the cationic lipids disclosed herein may be used as a means of improving (*e.g.*, reducing) the toxicity or otherwise conferring one or more desired properties to such enriched liposomal composition (*e.g.*, improved delivery of the encapsulated polynucleotides to one or more target cells and/or reduced in vivo toxicity of a liposomal composition). Accordingly, also contemplated are pharmaceutical compositions, and in particular liposomal compositions, that comprise one or more of the cationic lipids disclosed herein.

[0501] Thus, in certain embodiments, the compounds of the invention as described herein may be used as a component of a liposomal composition to facilitate or enhance the delivery and release of encapsulated materials (*e.g.*, one or more therapeutic agents) to one or more target cells (*e.g.*, by permeating or fusing with the lipid membranes of such target cells).

[0502] As used herein, liposomal delivery vehicles, *e.g.*, lipid nanoparticles, are usually characterized as microscopic vesicles having an interior aqueous space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphiphilic polymers and surfactants (*e.g.*, polymersomes, niosomes, *etc.*). In the context of the present invention, a liposomal delivery vehicle typically serves to transport a desired mRNA to a target cell or tissue.

[0503] In certain embodiments, such compositions (*e.g.*, liposomal compositions) are loaded with or otherwise encapsulate materials, such as for example, one or more biologically-active polynucleotides (*e.g.*, mRNA).

[0504] In embodiments, a composition (*e.g.*, a pharmaceutical composition) comprises an mRNA encoding a protein, encapsulated within a liposome. In embodiments, a liposome comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids, and wherein at least one cationic lipid is a compound of the invention as described herein. In embodiments, a composition comprises an mRNA encoding for a protein (*e.g.*, any protein described herein). In embodiments, a composition comprises an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. In embodiments, a composition comprises an mRNA encoding for ornithine transcarbamylase (OTC) protein.

[0505] In embodiments, a composition (*e.g.*, a pharmaceutical composition) comprises a nucleic acid encapsulated within a liposome, wherein the liposome comprises a compound described herein.

[0506] In embodiments, a nucleic acid is an mRNA encoding a peptide or protein. In embodiments, an mRNA encodes a peptide or protein for use in the delivery to or treatment of the lung of a subject or a lung cell (*e.g.*, an mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein). In embodiments, an mRNA encodes a peptide or protein for use in the delivery to or treatment of the liver of a subject or a liver cell (*e.g.*, an mRNA encodes ornithine transcarbamylase (OTC) protein). Still other exemplary mRNAs are described herein.

[0507] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net positive charge.

[0508] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net negative charge.

[0509] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net neutral charge.

[0510] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein.

[0511] For example, the amount of a compound of the invention as described herein in a composition can be described as a percentage ("wt%") of the combined dry weight of all lipids of a composition (*e.g.*, the combined dry weight of all lipids present in a liposomal composition).

[0512] In embodiments of the pharmaceutical compositions described herein, a compound of the invention as described herein is present in an amount that is about 0.5 wt% to about 30 wt% (*e.g.*, about 0.5 wt% to about 20 wt%) of the combined dry weight of all lipids present in a composition (*e.g.*, a liposomal composition).

[0513] In embodiments, a compound of the invention as described herein is present in an amount that is about 1 wt% to about 30 wt%, about 1 wt% to about 20 wt%, about 1 wt% to about 15 wt%, about 1 wt% to about 10 wt%, or about 5 wt% to about 25 wt% of the combined dry weight of all lipids present in a composition (*e.g.*, a liposomal composition). In embodiments, a compound of the invention as described herein is present in an amount that is about 0.5 wt% to about 5 wt%, about 1 wt% to about 10 wt%, about 5 wt% to about 20 wt%, or about 10 wt% to about 20 wt% of the combined dry weight of all lipids present in a composition such as a liposomal delivery vehicle.

[0514] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is at least about 5 wt%, about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, about 55 wt%, about 60 wt%, about 65 wt%, about 70 wt%, about 75 wt%, about 80 wt%, about 85 wt%, about 90 wt%, about 95 wt%, about 96 wt%, about 97 wt%, about 98 wt%, or about 99 wt% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0515] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is no more than about 5 wt%, about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, about 55 wt%, about 60 wt%, about 65 wt%, about 70 wt%, about 75 wt%, about 80 wt%, about 85 wt%, about 90 wt%, about 95 wt%, about 96 wt%, about 97 wt%, about 98 wt%, or about 99 wt% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0516] In embodiments, a composition (*e.g.*, a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.1 wt% to about 20 wt% (*e.g.*, about 0.1 wt% to about 15 wt%) of a compound described herein. In embodiments, a delivery vehicle (*e.g.*, a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.5 wt%, about 1 wt%, about 3 wt%, about 5 wt%, or about 10 wt% of a compound described herein. In embodiments, a delivery vehicle (*e.g.*,

a liposomal delivery vehicle such as a lipid nanoparticle) comprises up to about 0.5 wt%, about 1 wt%, about 3 wt%, about 5 wt%, about 10 wt%, about 15 wt%, or about 20 wt% of a compound described herein. In embodiments, the percentage results in an improved beneficial effect (*e.g.*, improved delivery to targeted tissues such as the liver or the lung).

- 5 **[0517]** The amount of a compound of the invention as described herein in a composition also can be described as a percentage ("mol%") of the combined molar amounts of total lipids of a composition (*e.g.*, the combined molar amounts of all lipids present in a liposomal delivery vehicle).

- 10 **[0518]** In embodiments of pharmaceutical compositions described herein, a compound of the invention as described herein is present in an amount that is about 0.5 mol% to about 50 mol% (*e.g.*, about 0.5 mol% to about 20 mol%) of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle.

- 15 **[0519]** In embodiments, a compound of the invention as described herein is present in an amount that is about 0.5 mol% to about 5 mol%, about 1 mol% to about 10 mol%, about 5 mol% to about 20 mol%, about 10 mol% to about 20 mol%, about 15 mol% to about 30 mol%, about 20 mol% to about 35 mol%, about 25 mol% to about 40 mol%, about 30 mol% to about 45 mol%, about 35 mol% to about 50 mol%, about 40 mol% to about 55 mol %, or about 45 mol% to about 60 mol% of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle. In embodiments, a compound of the invention as described herein is present in
20 an amount that is about 1 mol% to about 60 mol%, 1 mol% to about 50 mol%, 1 mol% to about 40 mol%, 1 mol% to about 30 mol%, about 1 mol% to about 20 mol%, about 1 mol% to about 15 mol%, about 1 mol% to about 10 mol%, about 5 mol% to about 55 mol%, about 5 mol% to about 45 mol%, about 5 mol% to about 35 mol% or about 5 mol% to about 25 mol% of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle

- 25 **[0520]** In certain embodiments, a compound of the invention as described herein can comprise from about 0.1 mol% to about 50 mol%, or from 0.5 mol% to about 50 mol%, or from about 1 mol% to about 50 mol%, or from about 5 mol% to about 50 mol%, or from about 10 mol% to about 50 mol%, or from about 15 mol% to about 50 mol%, or from about 20 mol% to about 50 mol%, or from about 25 mol% to about 50 mol%, or from about 30 mol% to about 50 mol%, of the
30 total amount of lipids in a composition (*e.g.*, a liposomal delivery vehicle).

[0521] In certain embodiments, a compound of the invention as described herein can comprise greater than about 0.1 mol%, or greater than about 0.5 mol%, or greater than about 1 mol%,

greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol% of the total amount of lipids in the lipid nanoparticle.

5 [0522] In certain embodiments, a compound as described can comprise less than about 60 mol%, or less than about 55 mol%, or less than about 50 mol%, or less than about 45 mol%, or less than about 40 mol%, or less than about 35 mol %, less than about 30 mol%, or less than about 25 mol%, or less than about 10 mol%, or less than about 5 mol%, or less than about 1 mol% of the total amount of lipids in a composition (*e.g.*, a liposomal delivery vehicle).

10 [0523] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is at least about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, about 30 mol%, about 35 mol%, about 40 mol%, about 45 mol%, about 50 mol%, about 55 mol%, about 60 mol%, about 65 mol%, about 70 mol%, about 75 mol%, about 80 mol%, about 85 mol%, about 90 mol%, about 95 mol%, about 96 mol%, about 97 mol%, about 98 mol%, or about 99 mol% of the combined molar amounts of total lipids in a composition (*e.g.*, a liposomal composition).

15 [0524] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is no more than about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, about 30 mol%, about 35 mol%, about 40 mol%, about 45 mol%, about 50 mol%, about 55 mol%, about 60 mol%, about 65 mol%, about 70 mol%, about 75 mol%, about 20 80 mol%, about 85 mol%, about 90 mol%, about 95 mol%, about 96 mol%, about 97 mol%, about 98 mol%, or about 99 mol% of the combined molar amounts of total lipids in a composition (*e.g.*, a liposomal composition).

[0525] In embodiments, the percentage results in an improved beneficial effect (*e.g.*, improved delivery to targeted tissues such as the liver or the lung).

25 [0526] In a typical embodiment, a composition of the invention (*e.g.*, a liposomal composition) comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, and one or more PEG-modified lipids, wherein at least one cationic lipid is a compound of the invention as described herein. For example, a composition suitable for practicing the invention has four lipid components comprising a compound of the invention as described herein as the cationic lipid component, a non-cationic lipid, a cholesterol-based lipid 30 and a PEG-modified lipid. The non-cationic lipid may be DOPE or DEPE. The cholesterol-based lipid may be cholesterol. The PEG-modified lipid may be DMG-PEG2K.

[0527] In further embodiments, pharmaceutical (*e.g.*, liposomal) compositions comprise one or more of a PEG-modified lipid, a non-cationic lipid and a cholesterol lipid. In other embodiments, such pharmaceutical (*e.g.*, liposomal) compositions comprise: one or more PEG-modified lipids; one or more non-cationic lipids; and one or more cholesterol lipids. In yet further embodiments, such pharmaceutical (*e.g.*, liposomal) compositions comprise: one or more PEG-modified lipids and one or more cholesterol lipids.

[0528] In embodiments, a composition (*e.g.*, lipid nanoparticle) that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein and one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid.

[0529] In embodiments, a composition (*e.g.*, lipid nanoparticle) that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compound of the invention as described herein; one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid; and further comprises a cholesterol-based lipid. Typically, such a composition has four lipid components comprising a compound of the invention as described herein as the cationic lipid component, a non-cationic lipid (*e.g.*, DOPE), a cholesterol-based lipid (*e.g.*, cholesterol) and a PEG-modified lipid (*e.g.*, DMG-PEG2K).

[0530] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein, as well as one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, a PEGylated lipid, and a cholesterol-based lipid.

[0531] According to various embodiments, the selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells, the characteristics of the mRNA to be delivered. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus, the molar ratios may be adjusted accordingly.

Synthesis of Lipid Nanoparticles

[0532] In embodiments, lipids described herein can be used in the preparation of lipid nanoparticles according to methods known in the art. For example, suitable methods include

methods described in International Publication No. WO 2018/089801, which is hereby incorporated by reference in its entirety.

- [0533] One exemplary process for lipid nanoparticle formulation is **Process A** of WO 2018/089801 (see, e.g., Example 1 and Figure 1 of WO 2018/089801). **Process A** ("A") relates to a conventional method of encapsulating mRNA by mixing mRNA with a mixture of lipids, without first pre-forming the lipids into lipid nanoparticles. In an exemplary process, an ethanol lipid solution and an aqueous buffered solution of mRNA are prepared separately. A solution of mixture of lipids (cationic lipid, helper lipids, zwitterionic lipids, PEG lipids etc.) is prepared by dissolving lipids in ethanol. The mRNA solution is prepared by dissolving the mRNA in citrate buffer, resulting in mRNA at a concentration of 0.0833mg/ml in citrate buffer with a pH of 4.5. Then, these two solutions are mixed using a pump system. In some instances, the two solutions are mixed using a gear pump system. In certain embodiments, the two solutions are mixing using a 'T' junction (or "Y" junction). The mixture is then purified by diafiltration with a TFF process. The resultant formulation is concentrated and stored at 2-8 °C until further use.
- [0534] A second exemplary process for lipid nanoparticle formulation is **Process B** of WO 2018/089801 (see, e.g., Example 2 and Figure 2 of WO 2018/089801). **Process B** ("B") refers to a process of encapsulating messenger RNA (mRNA) by mixing pre-formed lipid nanoparticles with mRNA. A range of different conditions, such as varying temperatures (i.e., heating or not heating the mixture), buffers, and concentrations, may be employed in Process B. In an exemplary process, lipids dissolved in ethanol and citrate buffer are mixed using a pump system. The instantaneous mixing of the two streams results in the formation of empty lipid nanoparticles, which is a self-assembly process. The resultant formulation mixture comprises empty lipid nanoparticles in citrate buffer containing alcohol. The formulation is then subjected to a TFF purification process wherein buffer exchange occurs. The resulting suspension of pre-formed empty lipid nanoparticles is then mixed with mRNA using a pump system. For certain cationic lipids, heating the solution post-mixing can result in a higher percentage of lipid nanoparticles containing mRNA and a higher total yield of mRNA.

Cationic Lipids

- [0535] In addition to any of the compounds of the invention as described herein, a composition may comprise one or more additional cationic lipids.

[0536] In some embodiments, liposomes may comprise one or more additional cationic lipids. As used herein, the phrase “cationic lipid” refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have been described in the literature, many of which are commercially available.

- 5 [0537] Suitable additional cationic lipids for use in the compositions include the cationic lipids as described in the literature.

Helper Lipids

- [0538] Compositions (*e.g.*, liposomal compositions) may also comprise one or more helper lipids. Such helper lipids include non-cationic lipids. As used herein, the phrase “non-cationic lipid” refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase “anionic lipid” refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC),
15 dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE), palmitoyl-oleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine
20 (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), or a mixture thereof. A non-cationic or helper lipid suitable for practicing the invention is dioleoylphosphatidylethanolamine (DOPE). Alternatively, 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE) can be used as a non-cationic or helper lipid.

- 25 [0539] In some embodiments, a non-cationic lipid is a neutral lipid, *i.e.*, a lipid that does not carry a net charge in the conditions under which the composition is formulated and/or administered.

- [0540] In some embodiments, a non-cationic lipid may be present in a molar ratio (mol%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%,
30 about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a molar ratio (mol%) of about 5% to about 90%, about 5% to about 70%,

about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%.

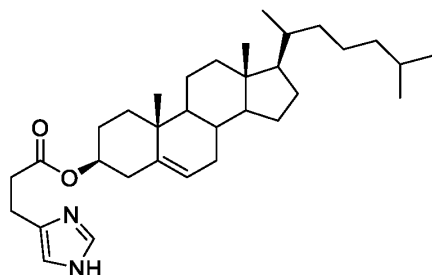
[0541] In some embodiments, a non-cationic lipid may be present in a weight ratio (wt%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a weight ratio (wt%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about 40 wt%. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about 40 wt%.

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Cholesterol-based Lipids

[0542] In some embodiments, a composition (*e.g.*, a liposomal composition) comprises one or more cholesterol-based lipids. For example, a suitable cholesterol-based lipid for practicing the invention is cholesterol. Other suitable cholesterol-based lipids include, for example, DC-Chol

(N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, *et al.* Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf *et al.* BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or imidazole cholesterol ester (ICE), which has the following structure,



("ICE").

5 [0543] In some embodiments, a cholesterol-based lipid may be present in a molar ratio (mol%) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage of
10 cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%.

[0544] In some embodiments, a cholesterol-based lipid may be present in a weight ratio (wt%) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In
15 some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about
20 40 wt%.

PEGylated Lipids

[0545] In some embodiments, a composition (*e.g.*, a liposomal composition) comprises one or more further PEGylated lipids. A suitable PEG-modified or PEGylated lipid for practicing the invention is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2K).

25 [0546] For example, the use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-octanoyl-sphingosine-1-

[succinyl(methoxy polyethylene glycol)-2000] (C8 PEG-2000 ceramide) is also contemplated by the present invention in combination with one or more of compounds of the invention as described herein and, in some embodiments, other lipids together which comprise the liposome. In some embodiments, particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (*e.g.*, C₁₄ or C₁₈).

[0547] Contemplated further PEG-modified lipids (also referred to herein as a PEGylated lipid, which term is interchangeable with PEG-modified lipid) include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ length. In some embodiments, a PEG-modified or PEGylated lipid is PEGylated cholesterol or PEG-2K. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target cell, (Klibanov *et al.* (1990) FEBS Letters, 268 (1): 235-237), or they may be selected to rapidly exchange out of the formulation in vivo (see U.S. Pat. No. 5,885,613).

[0548] Further PEG-modified phospholipid and derivatized lipids of the present invention may be present in a molar ratio (mol%) from about 0% to about 10%, about 0.5% to about 10%, about 1% to about 10%, about 2% to about 10%, or about 3% to about 5% of the total lipid present in the composition (*e.g.*, a liposomal composition).

Pharmaceutical Formulations and Therapeutic Uses

[0549] Compounds of the invention as described herein may be used in the preparation of compositions (*e.g.*, to construct liposomal compositions) that facilitate or enhance the delivery and release of encapsulated materials (*e.g.*, one or more therapeutic polynucleotides) to one or more target cells (*e.g.*, by permeating or fusing with the lipid membranes of such target cells).

[0550] For example, when a liposomal composition (*e.g.*, a lipid nanoparticle) comprises or is otherwise enriched with one or more of the compounds disclosed herein, the phase transition in the lipid bilayer of the one or more target cells may facilitate the delivery of the encapsulated materials (*e.g.*, one or more therapeutic polynucleotides encapsulated in a lipid nanoparticle) into the one or more target cells.

[0551] Similarly, in certain embodiments compounds of the invention as described herein may be used to prepare liposomal vehicles that are characterized by their reduced toxicity *in vivo*. In certain embodiments, the reduced toxicity is a function of the high transfection efficiencies associated with the compositions disclosed herein, such that a reduced quantity of such

composition may administered to the subject to achieve a desired therapeutic response or outcome.

[0552] Thus, pharmaceutical formulations comprising a compound described and nucleic acids provided by the present invention may be used for various therapeutic purposes. To facilitate
5 delivery of nucleic acids *in vivo*, a compound described herein and nucleic acids can be formulated in combination with one or more additional pharmaceutical carriers, targeting ligands or stabilizing reagents. In some embodiments, a compound described herein can be formulated via pre-mixed lipid solution. In other embodiments, a composition comprising a compound described herein can be formulated using post-insertion techniques into the lipid membrane of the
10 nanoparticles. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

[0553] Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous,
15 intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, or intranasal. In particular embodiments, the intramuscular administration is to a muscle selected from the group consisting of skeletal muscle, smooth muscle and cardiac muscle. In some embodiments the administration results in delivery of the nucleic acids to a muscle cell. In some embodiments the administration results in delivery of the nucleic acids to a hepatocyte
20 (*i.e.*, liver cell).

[0554] A common route for administering a liposomal composition of the invention may be intravenous delivery, in particular when treating metabolic disorders, especially those affecting the liver (e.g., ornithine transcarbamylase (OTC) deficiency). Alternatively, depending on the disease or disorder to be treated, the liposomal composition may be administered via pulmonary
25 delivery (e.g., for the treatment of cystic fibrosis). For vaccination, a liposomal composition of the invention is typically administered intramuscularly. Diseases or disorders affecting the eye may be treated by administering a liposomal composition of the invention intravitreally.

[0555] Alternatively or additionally, pharmaceutical formulations of the invention may be administered in a local rather than systemic manner, for example, via injection of the
30 pharmaceutical formulation directly into a targeted tissue (e.g., in a sustained release formulation). Local delivery can be affected in various ways, depending on the tissue to be targeted. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include,

- but are not limited to the liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid. In embodiments, the tissue to be targeted in the liver. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the
- 5 site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection.
- 10 **[0556]** Compositions described herein can comprise mRNA encoding peptides including those described herein (*e.g.*, a polypeptide such as a protein).
- [0557]** In embodiments, a mRNA encodes a polypeptide.
- [0558]** In embodiments, a mRNA encodes a protein.
- [0559]** Exemplary peptides encoded by mRNA (*e.g.*, exemplary proteins encoded by mRNA) are
- 15 described herein.
- [0560]** The present invention provides methods for delivering a composition having full-length mRNA molecules encoding a peptide or protein of interest for use in the treatment of a subject, *e.g.*, a human subject or a cell of a human subject or a cell that is treated and delivered to a human subject.
- 20 **[0561]** In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or protein for use in the delivery of or treatment with a vaccine for a subject or a cell of a subject. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from an infectious agent, such as a virus.
- 25 **[0562]** In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen associated with a cancer of a subject or identified from a cancer cell of a subject. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen determined from a subject's own cancer cell, *i.e.*, to provide a
- 30 personalized cancer vaccine.

[0563] In embodiments, a composition comprises an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein.

[0564] In embodiments, a composition comprises an mRNA encoding for ornithine transcarbamylase (OTC) protein.

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Delivery Methods

[0565] The route of delivery used in the methods of the invention allows for non-invasive, self-administration of the compounds of the invention. In some embodiments, the methods involve intratracheal or pulmonary administration by aerosolization, nebulization, or instillation of a composition comprising mRNA encoding a therapeutic protein in a suitable transfection or lipid carrier vehicles as described above. In some embodiments, the protein is encapsulated with a liposome. In some embodiments, the liposome comprises a lipid, which is a compound of the invention. As used herein below, administration of a compound of the invention includes administration of a composition comprising a compound of the invention.

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[0566] Although the local cells and tissues of the lung represent a potential target capable of functioning as a biological depot or reservoir for production and secretion of the protein encoded by the mRNA, applicants have discovered that administration of the compounds of the invention to the lung via aerosolization, nebulization, or instillation results in the distribution of even non-secreted proteins outside the lung cells. Without wishing to be bound by any particular theory, it is contemplated that nanoparticle compositions of the invention pass, through the lung airway-blood barrier, resulting in translation of the intact nanoparticle to non-lung cells and tissues, such as, *e.g.*, the heart, the liver, the spleen, where it results in the production of the encoded protein in these non-lung tissues. Thus, the utility of the compounds of the invention and methods of the invention extend beyond production of therapeutic protein in lung cells and tissues of the lung

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and can be used to delivery to non-lung target cells and/or tissues. They are useful in the management and treatment of a large number of diseases, and in particular peripheral diseases which result from both secreted and non-secreted protein and/or enzyme deficiencies (*e.g.*, one or more lysosomal storage disorders). In certain embodiments, the compounds of the invention, used in the methods of the invention result in the distribution of the mRNA encapsulated

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nanoparticles and production of the encoded protein in the liver, spleen, heart, and/or other non-lung cells. For example, administration of the compounds of the invention, by aerosolization, nebulization, or instillation to the lung will result in the composition itself and its protein product

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(*e.g.*, functional beta galactosidase protein) will be detectable in both the local cells and tissues of the lung, as well as in peripheral target cells, tissues and organs as a result of translocation of the mRNA and delivery vehicle to non-lung cells.

[0567] In certain embodiments, the compounds of the invention may be employed in the methods of the invention to specifically target peripheral cells or tissues. Following the pulmonary delivery, it is contemplated the compounds of the invention cross the lung airway-blood barrier and distribute into cells other than the local lung cells. Accordingly, the compounds disclosed herein may be administered to a subject by way of the pulmonary route of administration, using a variety of approach known by those skilled in the art (*e.g.*, by inhalation), and distribute to both the local target cells and tissues of the lung, as well as in peripheral non-lung cells and tissues (*e.g.*, cells of the liver, spleen, kidneys, heart, skeletal muscle, lymph nodes, brain, cerebrospinal fluid, and plasma). As a result, both the local cells of the lung and the peripheral non-lung cells can serve as biological reservoirs or depots capable of producing and/or secreting a translation product encoded by one or more polynucleotides. Accordingly, the present invention is not limited to the treatment of lung diseases or conditions, but rather can be used as a non-invasive means of facilitating the delivery of polynucleotides, or the production of enzymes and proteins encoded thereby, in peripheral organs, tissues and cells (*e.g.*, hepatocytes) which would otherwise be achieved only by systemic administration. Exemplary peripheral non-lung cells include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

[0568] Following administration of the composition to the subject, the protein product encoded by the mRNA (*e.g.*, a functional protein or enzyme) is detectable in the peripheral target tissues for at least about one to seven days or longer following administration of the compound to the subject. The amount of protein product necessary to achieve a therapeutic effect will vary depending on the condition being treated, the protein encoded, and the condition of the patient. For example, the protein product may be detectable in the peripheral target tissues at a concentration (*e.g.*, a therapeutic concentration) of at least 0.025-1.5 µg/ml (*e.g.*, at least 0.050 µg/ml, at least 0.075 µg/ml, at least 0.1 µg/ml, at least 0.2 µg/ml, at least 0.3 µg/ml, at least 0.4 µg/ml, at least 0.5 µg/ml, at least 0.6 µg/ml, at least 0.7 µg/ml, at least 0.8 µg/ml, at least 0.9 µg/ml, at least 1.0 µg/ml, at least 1.1 µg/ml, at least 1.2 µg/ml, at least 1.3 µg/ml, at least 1.4

µg/ml, or at least 1.5 µg/ml), for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 days or longer following administration of the compound to the subject.

[0569] It has been demonstrated that nucleic acids can be delivered to the lungs by intratracheal administration of a liquid suspension of the compound and inhalation of an aerosol mist produced by a liquid nebulizer or the use of a dry powder apparatus such as that described in U.S. patent 5,780,014, incorporated herein by reference.

[0570] In certain embodiments, the compounds of the invention may be formulated such that they may be aerosolized or otherwise delivered as a particulate liquid or solid prior to or upon administration to the subject. Such compounds may be administered with the assistance of one or more suitable devices for administering such solid or liquid particulate compositions (such as, *e.g.*, an aerosolized aqueous solution or suspension) to generate particles that are easily respirable or inhalable by the subject. In some embodiments, such devices (*e.g.*, a metered dose inhaler, jet-nebulizer, ultrasonic nebulizer, dry-powder-inhalers, propellant-based inhaler or an insufflator) facilitate the administration of a predetermined mass, volume or dose of the compositions (*e.g.*, about 0.5 mg/kg of mRNA per dose) to the subject. For example, in certain embodiments, the compounds of the invention are administered to a subject using a metered dose inhaler containing a suspension or solution comprising the compound and a suitable propellant. In certain embodiments, the compounds of the invention may be formulated as a particulate powder (*e.g.*, respirable dry particles) intended for inhalation. In certain embodiments, compositions of the invention formulated as respirable particles are appropriately sized such that they may be respirable by the subject or delivered using a suitable device (*e.g.*, a mean D50 or D90 particle size less than about 500µm, 400µm, 300µm, 250µm, 200µm, 150µm, 100µm, 75µm, 50µm, 25µm, 20µm, 15µm, 12.5µm, 10µm, 5µm, 2.5µm or smaller). In yet other embodiments, the compounds of the invention are formulated to include one or more pulmonary surfactants (*e.g.*, lamellar bodies). In some embodiments, the compounds of the invention are administered to a subject such that a concentration of at least 0.05 mg/kg, at least 0.1 mg/kg, at least 0.5 mg/kg, at least 1.0 mg/kg, at least 2.0 mg/kg, at least 3.0 mg/kg, at least 4.0 mg/kg, at least 5.0 mg/kg, at least 6.0 mg/kg, at least 7.0 mg/kg, at least 8.0 mg/kg, at least 9.0 mg/kg, at least 10 mg/kg, at least 15 mg/kg, at least 20 mg/kg, at least 25 mg/kg, at least 30 mg/kg, at least 35 mg/kg, at least 40 mg/kg, at least 45 mg/kg, at least 50 mg/kg, at least 55 mg/kg, at least 60 mg/kg, at least 65 mg/kg, at least 70 mg/kg, at least 75 mg/kg, at least 80 mg/kg, at least 85 mg/kg, at least 90 mg/kg, at least 95 mg/kg, or at least 100 mg/kg body weight is administered in

a single dose. In some embodiments, the compounds of the invention are administered to a subject such that a total amount of at least 0.1 mg, at least 0.5 mg, at least 1.0 mg, at least 2.0 mg, at least 3.0 mg, at least 4.0 mg, at least 5.0 mg, at least 6.0 mg, at least 7.0 mg, at least 8.0 mg, at least 9.0 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 35 mg, at least 40 mg, at least 45 mg, at least 50 mg, at least 55 mg, at least 60 mg, at least 65 mg, at least 70 mg, at least 75 mg, at least 80 mg, at least 85 mg, at least 90 mg, at least 95 mg or at least 100 mg mRNA is administered in one or more doses.

Examples

10 [0571] Abbreviations

DCM = dichloromethane

DIPEA = N,N-Diisopropylethylamine

DM = demineralized

DMAP = 4-Dimethylaminopyridine

15 EDC-HCl = *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride

EDCI = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

ELSD = evaporative light-scattering detection

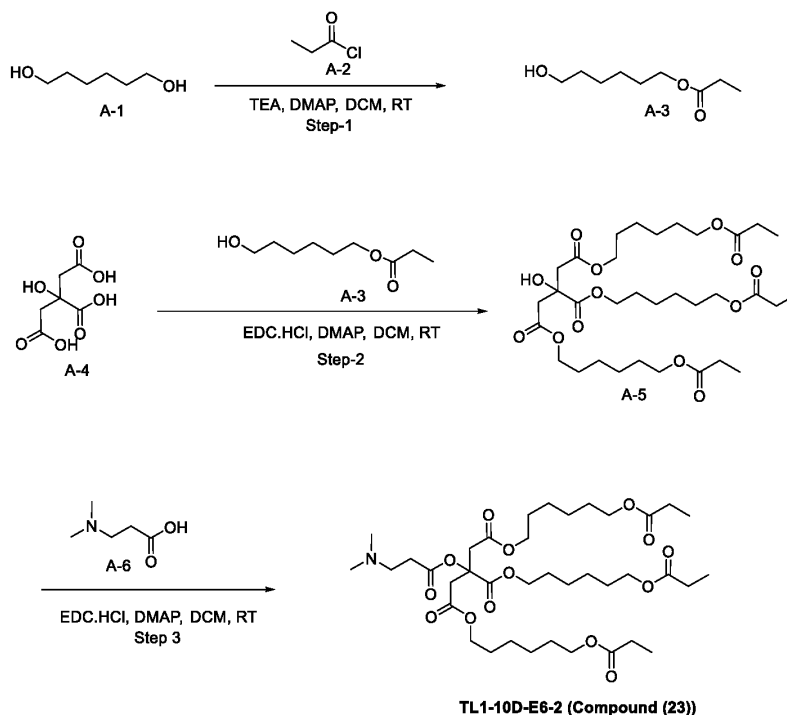
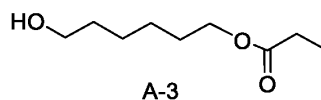
EtOAc = ethyl acetate

h = hour

20 r.t. = room temperature

SM = starting material

TLC = thin layer chromatography

Example 1. Synthesis of JC-TL1-10D-E6-2 (Compound (13))**Synthetic Scheme A****Intermediate [A-3]:**

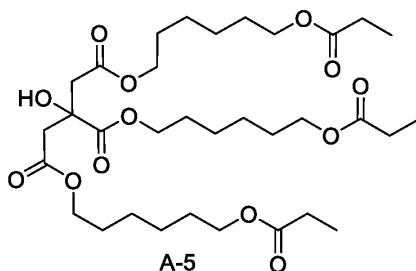
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[0572] To a stirred solution of hexane-1,6-diol **[A-1]** (10 g, 0.085 mol) and triethylamine (42.9 g, 0.45 mol) in dichloromethane (150 mL) was cooled to 0 °C, 4-(dimethylamino)pyridin-1-ium (1.03 g, 0.0085 mol) was added followed by propionyl chloride **[A-2]** (3.93 g, 0.042 mol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by TLC (SM was consumed). Reaction mixture was quenched by cold saturated NaHCO₃ upto pH 7, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-20 % ethyl acetate in hexane), to give the desired 6-hydroxyhexyl propionate **[A-3]** (4.5 g, 30 %, Yield) as a light yellow oil.

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[0573] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 5.6 Hz, 2H), 3.64 (br, 2H), 2.32 (q, J = 7.6 Hz, 2H), 1.67-1.60 (m, 2H), 1.58-1.54 (m, 2H), 1.44-1.37 (m, 4H), 1.13 (t, J = 7.6 Hz, 3H).

Intermediate [A-5]:



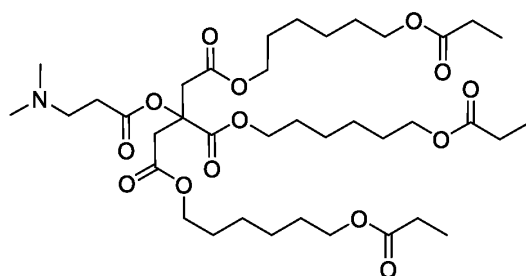
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[0574] To a stirred solution of 6-hydroxyhexyl propionate [A-3] (4.5 g, 0.026 mol) and citric acid [A-4] (1.25 g, 0.0065 mol) in dichloromethane (100 mL) was cooled to 0 °C, EDC.HCl (4.95 g, 0.026 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.78 g, 0.0065 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (50 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product tris(6-(propionyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [A-5] (0.57 g, 13 %, Yield) as a pale yellow liquid.

15 [0575] ^1H NMR (400 MHz, CDCl_3): δ 4.21 (t, J = 6.8 Hz, 2H), 4.12-4.04 (m, 10H), 2.88 (d, J = 15.6 Hz, 2H), 2.79 (d, J = 15.6 Hz, 2H), 2.32 (q, J = 7.6 Hz, 6H), 1.71-1.67 (m, 2H), 1.64-1.62 (m, 10H), 1.38-1.36 (m, 12H), 1.13 (t, J = 7.8 Hz, 9H).

[0576] *ELSD analysis*: Purity 98.66 %, Calculated $\text{C}_{33}\text{H}_{56}\text{O}_{13}$ = 660.37, Observed = 661.10 (m/z , $\text{M}+\text{H}^+$).

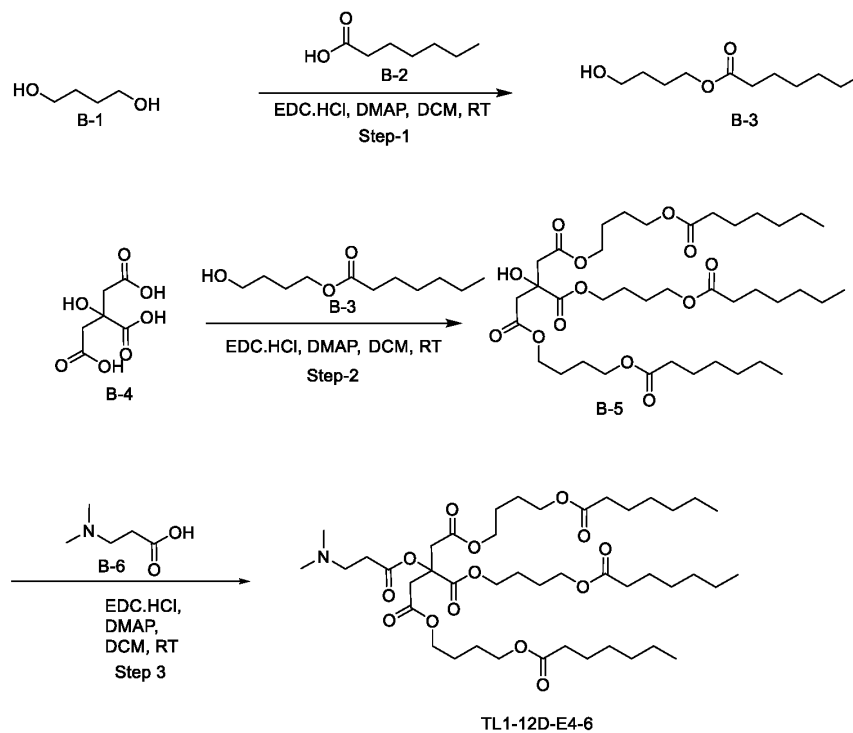
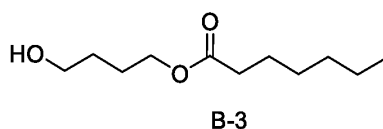
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JC-TL1-10D-E6-2 (Compound (13))**Compound (13)**

[0577] To a stirred solution of tris(6-(propionyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate **[A-5]** (0.57 g, 0.00086 mol) and 3-(dimethylamino)propanoic acid **[6]** (0.404 g, 0.00345 mol) in dichloromethane (15 mL) was cooled to 0 °C, EDC.HCl (0.662 g, 0.00345 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.105 g, 0.00086 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (20 mL) was added to the reaction mixture and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product tris(6-(propionyloxy)hexyl) 2-((3-(dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate **[JC-TL1-10D-E6-2]** (Compound (13)) (0.115 g, 17.4 %, Yield) as a yellow liquid.

[0578] ¹H NMR (400 MHz, DMSO-d₆): δ 4.05-3.97 (m, 12H), 3.16 (d, *J*= 15.2Hz, 2H), 3.08 (d, *J*= 15.2 Hz, 2H), 2.46-2.37 (m, 4H), 2.28 (q, *J*= 7.6 Hz, 6H), 2.10 (s, 6H), 1.56-1.53 (m, 12H), 1.31-1.30 (m, 12H), 1.01 (t, *J*= 6.8Hz, 9H).

[0579] *ELSD analysis*: Purity 99.66 %, Calculated C₃₈H₆₅NO₁₄ = 759.44, Observed = 760.50 (*m/z*, M+H⁺).

Example 2. Synthetic Protocol for M1266-J03983-009 (JC-TL1-12D-E4-6) (Compound (12))**Synthetic Scheme B****Intermediate [B-3]:**

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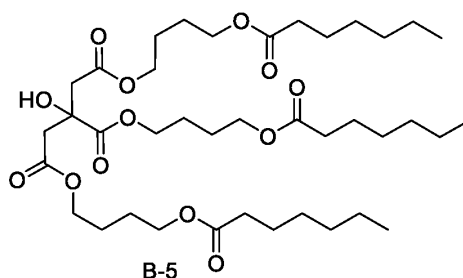
[0580] To a stirred solution of butane-1,4-diol **[B-1]** (10 g, 0.111 mol) and heptanoic acid **[B-2]** (14.4 g, 0.111 mol) in dichloromethane (200 mL) was cooled to 0 °C, EDC.HCl (25.5 g, 0.133 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (1.35 g, 0.011 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (200 mL) was added to the reaction mixture and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-20 % ethyl acetate in n-hexane) to obtain pure product 4-hydroxybutyl heptanoate **[B-3]** (6.2 g, 27 % Yield) as a yellow liquid.

10

Results:

[0581] ^1H NMR (400 MHz, CDCl_3): δ 4.09 (t, J = 6.0 Hz, 2H), 3.69-3.65 (m, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.73-1.59 (m, 6H), 1.28 (br, 6H), 0.87-0.85 (m, 3H).

5

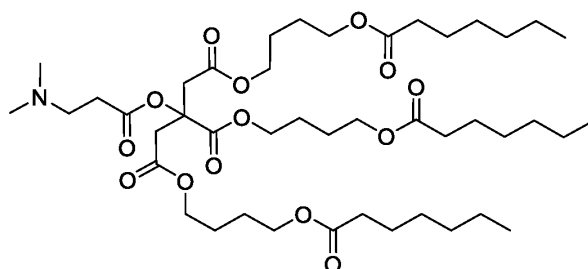
Intermediate [B-5]:

[0582] To a stirred solution of 4-hydroxybutyl heptanoate **[B-3]** (6.18 g, 0.0306 mol) and citric acid **[B-4]** (1.47 g, 0.00765 mol) in dichloromethane (150 mL) was cooled to 0 °C, EDC.HCl (5.84 g, 0.0306 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.9 g, 0.00765 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane) to obtain pure product tris(4-(heptanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate **[B-5]** (1.53 g, 27 % Yield) as a colourless liquid.

[0583] ^1H NMR (400 MHz, CDCl_3): δ 4.25 (t, J = 6.0 Hz, 2H), 4.14-4.07 (m, 10H), 2.88 (d, J = 15.6 Hz, 2H), 2.80 (d, J = 15.6 Hz, 2H), 2.29 (t, J = 7.6 Hz, 6H), 1.85-1.68 (m, 12H), 1.64-1.56 (m, 6H), 1.34-1.22 (m, 18H), 0.88 (t, J = 7.8 Hz, 9H).

[0584] *ELSD analysis:* Purity 98.99 %, Calculated $\text{C}_{39}\text{H}_{68}\text{O}_{13}$ = 744.46, Observed = 745.25 (m/z , $\text{M}+\text{H}^+$).

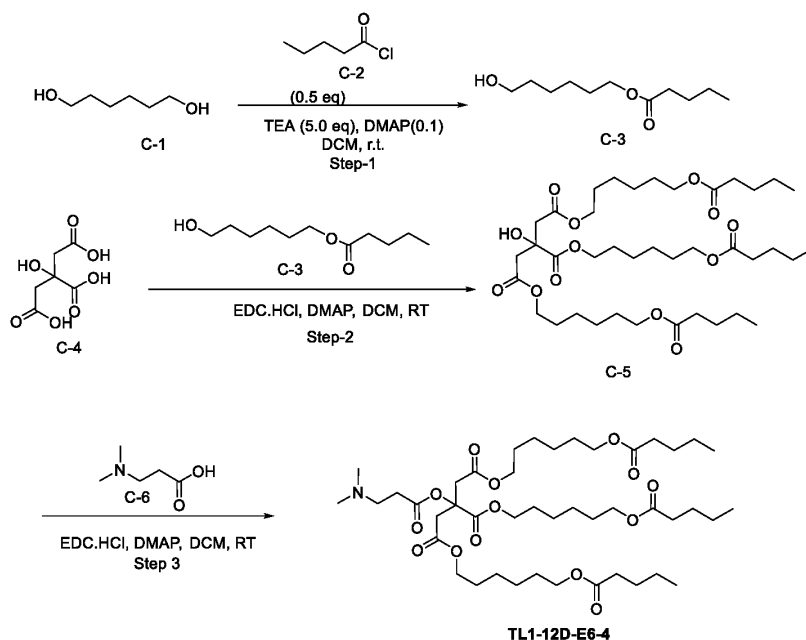
20

JC-TL1-12D-E4-6 (Compound (12))**(Compound (12))**

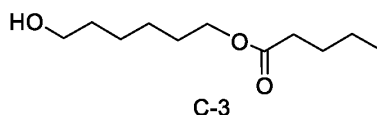
[0585] To a stirred solution of tris(4-(heptanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate **[5]** (1.53 g, 0.00205 mol) and 3-(dimethylamino)propanoic acid **[B-6]** (0.962 g, 0.00822 mol) in dichloromethane (30 mL) was cooled to 0 °C, EDC.HCl (1.56 g, 0.00822 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.251 g, 0.00205 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (20 mL) was added to the reaction mixture and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane) to obtain pure product tris(4-(heptanoyloxy)butyl) 2-((3-(dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate [JC-TL1-12D-E4-6] (Compound (12)) (0.3 g, 18 %, Yield) as a brown liquid.

[0586] ¹H NMR (400 MHz, CDCl₃): δ 4.18 (t, *J* = 6.0 Hz, 2H), 4.10-4.08 (m, 10H), 3.30 (d, *J* = 16.0 Hz, 2H), 3.23 (d, *J* = 15.6 Hz, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 6H), 2.21 (s, 6H), 1.72-1.67 (m, 12H), 1.64-1.59 (m, 7H), 1.34-1.25 (m, 17H), 0.88 (t, *J* = 6.4 Hz, 9H).

[0587] *ELSD analysis:* Purity 99.83 %, Calculated C₄₄H₇₇NO₁₄ = 843.53, Observed = 844.65 (m/z, M+H⁺).

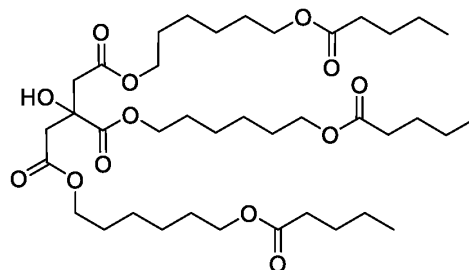
Example 3. Synthetic Protocol for M1266-J03983-027 (JC-TL1-12D-E6-4) (Compound (40))**Synthetic Scheme C**

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Intermediate [C-3]:

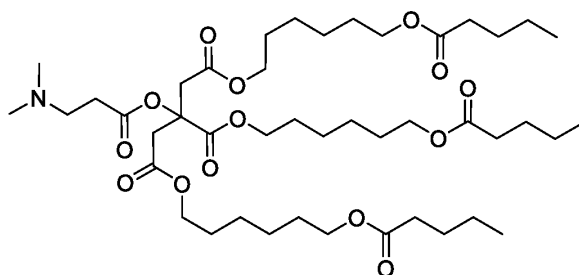
[0588] To a stirred solution of hexane-1,6-diol **[C-1]** (9.8 g, 0.08 mol) and triethylamine (41.8 g, 0.4 mol) in dichloromethane (150 mL) was cooled to 0 °C, 4-(dimethylamino)pyridin-1-ium (1.0 g, 0.008 mol) was added followed by pentanoyl chloride **[C-2]** (5.0 g, 0.04 mol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by TLC (SM was consumed). Reaction mixture was quenched by cold saturated NaHCO₃ upto pH 7, and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-20 % ethyl acetate in hexane), to give the desired 6-hydroxyhexyl pentanoate **[C-3]** (6.2 g, 36 %, Yield) as a light yellow oil.

[0589] ¹H NMR (400 MHz, CDCl₃): δ 4.06 (t, *J*= 6.8 Hz, 2H), 3.64 (t, *J*= 6.8 Hz, 2H), 2.29 (t, *J*= 7.2 Hz, 2H), 1.66-1.54 (m, 6H), 1.42-1.31 (m, 6H), 0.92-0.82 (m, 3H).

Intermediate [C-5]:**C-5**

- 5 **[0590]** To a stirred solution of 6-hydroxyhexyl pentanoate **[C-3]** (5.3 g, 0.026 mol) and Citric acid **[C-4]** (1.26 g, 0.0065 mol) in dichloromethane (100 mL) was cooled to 0 °C, EDC.HCl (5.02 g, 0.026 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.8 g, 0.0065 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x200
- 10 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product tris(6-(pentanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate **[C-5]** (1.61 g, 33 %, Yield) as a pale yellow liquid.

- [0591]** *ELSD analysis:* Purity 99.62 %, Calculated C₃₉H₆₈O₁₃ = 744.46, Observed = 767.20 (m/z, M+Na⁺).
- 15

JC-TL1-12D-E6-4 (Compound (40))**Compound (40)**

- [0592]** To a stirred solution of tris(6-(pentanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate **[C-5]** (1.61 g, 0.0022 mol) and 3-(dimethylamino)propanoic acid **[C-6]** (1.013 g, 0.0086 mol) in dichloromethane (30 mL) was cooled to 0 °C, EDC.HCl (1.69 g, 0.0086 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.26 g, 0.0022 mol) and stirred for 48 h at
- 20

room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (20 mL) was added to the reaction mixture and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product JC-TL1-12D-E6-4 (Compound (40)) (0.225 g, 12 %, Yield) as a pale yellow liquid.

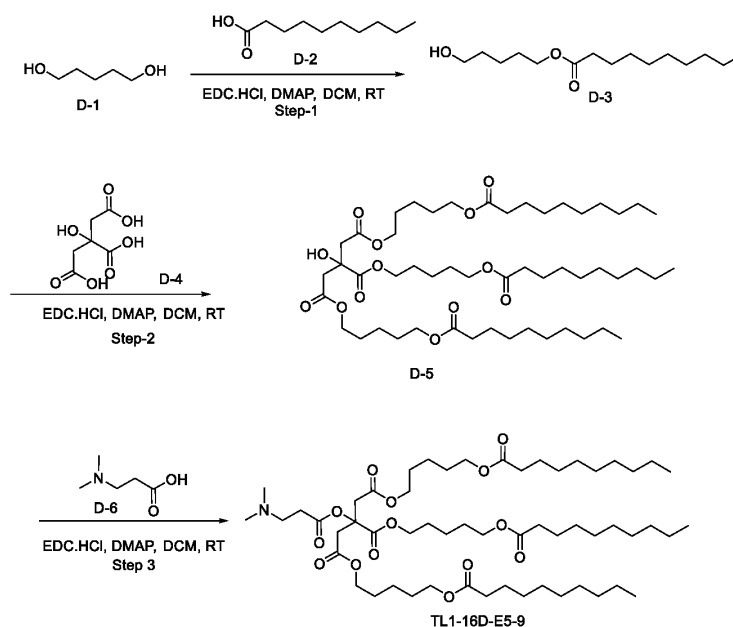
[0593] ¹H NMR (400 MHz, CDCl₃): δ 4.14 (t, *J* = 6.8 Hz, 2H), 4.08-4.03 (m, 10H), 3.30 (d, *J* = 15.6 Hz, 2H), 3.21 (d, *J* = 15.6 Hz, 2H), 2.60-2.44 (m, 4H), 2.29 (t, *J* = 7.6 Hz, 6H), 2.21 (s, 6H), 1.63-1.56 (m, 18H), 1.37-1.29 (m, 18H), 0.91 (t, *J* = 7.2 Hz, 9H).

[0594] *ELSD analysis*: Purity 98.58 %, Calculated C₄₄H₇₇NO₁₄ = 843.53, Observed = 844.25 (*m/z*, M+Na⁺).

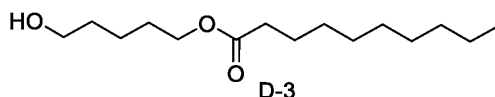
Example 4. Synthetic Protocol for M1266-J03983-025 (JC-TL1-16D-E5-9) (Compound (29))

15

Synthetic Scheme D



Intermediate [D-3]:

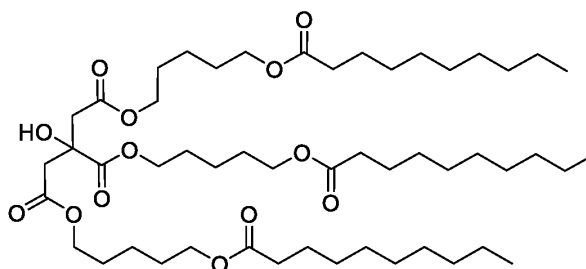


[0595] To a stirred solution of pentane-1,5-diol [D-1] (10 g, 0.0961 mol) and decanoic acid [D-2] (16.5 g, 0.0961 mol) in dichloromethane (200 mL) was cooled to 0 °C, EDC.HCl (22.0 g, 0.115 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (1.17 g, 0.009 mol) and stirred for 16 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed).

- 5 Water (100 mL) was added to the reaction mixture and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-20 % ethyl acetate in n-hexane), to obtain pure product 5-hydroxypentyl decanoate [D-3] (5.4 g, 22 % Yield) as a pale yellow liquid.

- 10 [0596] ¹H NMR (400 MHz, CDCl₃): δ 4.07 (t, *J*= 6.4 Hz, 2H), 3.68-3.63 (m, 2H), 2.28 (t, *J*= 7.6 Hz, 2H), 1.68-1.58 (m, 6H), 1.47-1.42 (m, 2H), 1.29-1.25 (m, 12H), 0.89-0.87 (m, 3H).

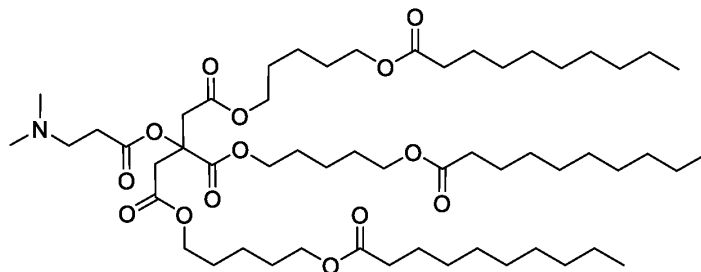
Intermediate [5]:



D-5

- 15 [0597] To a stirred solution of 5-hydroxypentyl decanoate [D-3] (5.37 g, 0.0208 mol) and Citric acid [D-4] (1.0 g, 0.0052 mol) in dichloromethane (100 mL) was cooled to 0 °C, EDC.HCl (4.0 g, 0.0208 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.635 g, 0.0052 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x200
- 20 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product tris(5-(decanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [D-5] (0.7 g, 14 % Yield) as a pale yellow liquid.

- [0598] ¹H NMR (400 MHz, CDCl₃): δ 4.22 (t, *J*= 6.8 Hz, 2H), 4.11-4.04 (m, 10H), 2.88 (d, *J*= 15.6 Hz, 2H), 2.79 (d, *J*= 15.6 Hz, 2H), 2.28 (t, *J*= 7.6 Hz, 6H), 1.74-1.59 (m, 16H), 1.46-1.36 (m, 6H), 1.29-1.26 (m, 38H), 0.89-0.86 (m, 9H).
- 25

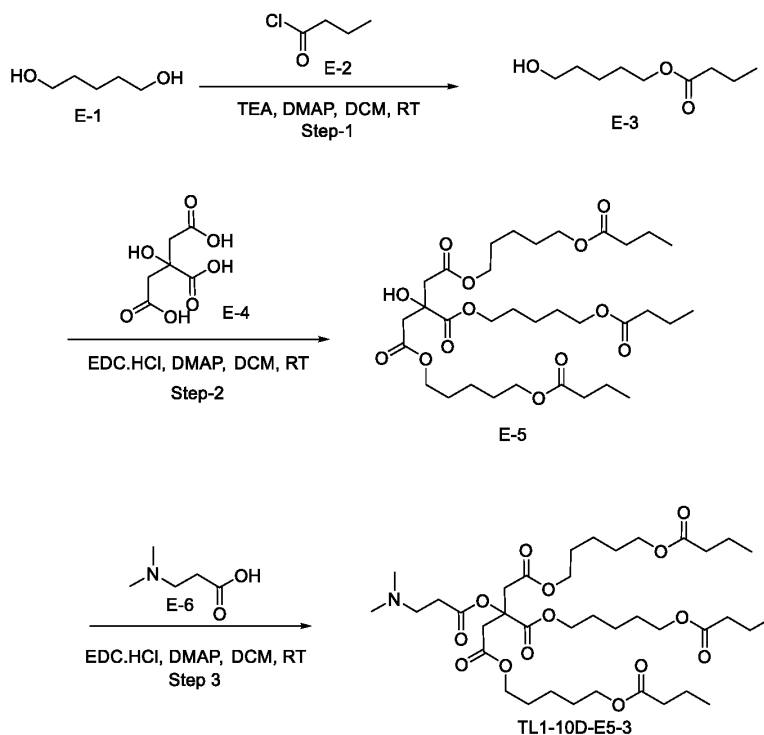
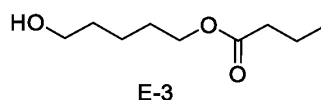
JC-TL1-16D-E5-9 (Compound (29))

(Compound (29))

- [0599]** To a stirred solution of tris(5-(decanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate **[D-5]** (0.7 g, 0.00078 mol) and 3-(dimethylamino)propanoic acid **[D-6]** (0.36 g, 0.00312 mol) in dichloromethane (20 mL) was cooled to 0 °C, EDC.HCl (0.59 g, 0.00312 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.09 g, 0.00078 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (10 mL) was added to the reaction mixture and extracted with DCM (3x25 mL). The resulting
- 10 organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product JC-TL1-16D-E5-9 (Compound (29)) (0.11 g, 14 %, Yield) as a colourless liquid.

- [0600]** ¹H NMR (400 MHz, CDCl₃): δ 4.15 (t, *J* = 6.8 Hz, 2H), 4.09-4.04 (m, 10H), 3.30 (d, *J* = 15.6 Hz, 2H), 3.22 (d, *J* = 15.6 Hz, 2H), 2.60-2.56 (m, 2H), 2.50-2.46 (m, 2H), 2.28 (t, *J* = 7.6 Hz, 6H), 2.22 (s, 6H), 1.69-1.59 (m, 18H), 1.44-1.37 (m, 6H), 1.29-1.26 (m, 36H), 0.87 (t, *J* = 6.4 Hz, 9H).
- 15

[0601] *ELSD analysis:* Purity 98.73 %, Calculated C₅₆H₁₀₁NO₁₄ = 1011.72, Observed = 1012.40 (m/z, M+H⁺).

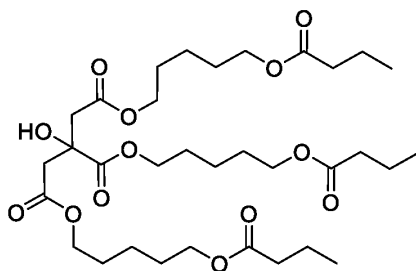
Example 5. Synthetic Protocol for M1266-J03984-031 (TL1-10D-E5-3) (Compound (38))**Synthetic Scheme E****Intermediate [E-3]:**

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- [0602]** To a stirred solution of pentane-1,5-diol **[E-1]** (10 g, 0.096 mol) and triethylamine (48.5 g, 0.48 mol) in dichloromethane (100 mL) was cooled to 0 °C, 4-(dimethylamino)pyridin-1-ium (1.17 g, 0.0096 mol) was added followed by butyryl chloride **[E-2]** (5.09 g, 0.048 mol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by TLC (SM was consumed). Reaction mixture was quenched by cold saturated NaHCO₃ upto pH 7, and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-20 % ethyl acetate in hexane), to give the desired 5-hydroxypentyl butyrate **[E-3]** (4.2 g, 25 %, Yield) as a colourless oil.

[0603] ^1H NMR (400 MHz, CDCl_3): δ 4.08 (t, J = 6.8 Hz, 2H), 3.68-3.63 (m, 2H), 2.28 (t, J = 7.2 Hz, 2H), 1.70-1.58 (m, 5H), 1.47-1.40 (m, 2H), 1.25 (t, J =5.2Hz, 1H), 0.94 (t, J = 7.6 Hz, 3H).

Intermediate [E-5]:



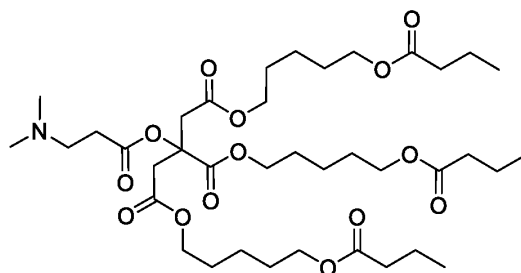
E-5

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[0604] To a stirred solution of 5-hydroxypentyl butyrate [E-3] (4.16 g, 0.0239 mol) and Citric acid [4] (1.15 g, 0.0059 mol) in dichloromethane (50 mL) was cooled to 0 °C, EDC.HCl (4.60 g, 0.0239 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.73 g, 0.0059 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product tris(5-(butyryloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [E-5] (1.05 g, 27 %, Yield) as a pale yellow liquid.

15 [0605] *ELSD analysis*: Purity 97.05 %, Calculated $\text{C}_{33}\text{H}_{56}\text{O}_{13}$ = 660.37, Observed = 661.10 (m/z , $\text{M}+\text{H}^+$).

JC-TL1-10D-E5-3 (Compound (38))



(Compound (38))

[0606] To a stirred solution of tris(5-(butyryloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [E-5] (1.0 g, 0.0015 mol) and 3-(dimethylamino)propanoic acid [E-6] (0.929 g, 0.006 mol) in dichloromethane (20 mL) was cooled to 0 °C, EDC.HCl (1.16 g, 0.006 mol), was added followed by

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- 4-(dimethylamino)pyridin-1-ium (0.184 g, 0.0015 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (20 mL) was added to the reaction mixture and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was
- 5 purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product JC-TL1-10D-E5-3 (Compound (38)) (0.410 g, 35 %, Yield) as a pale yellow liquid.

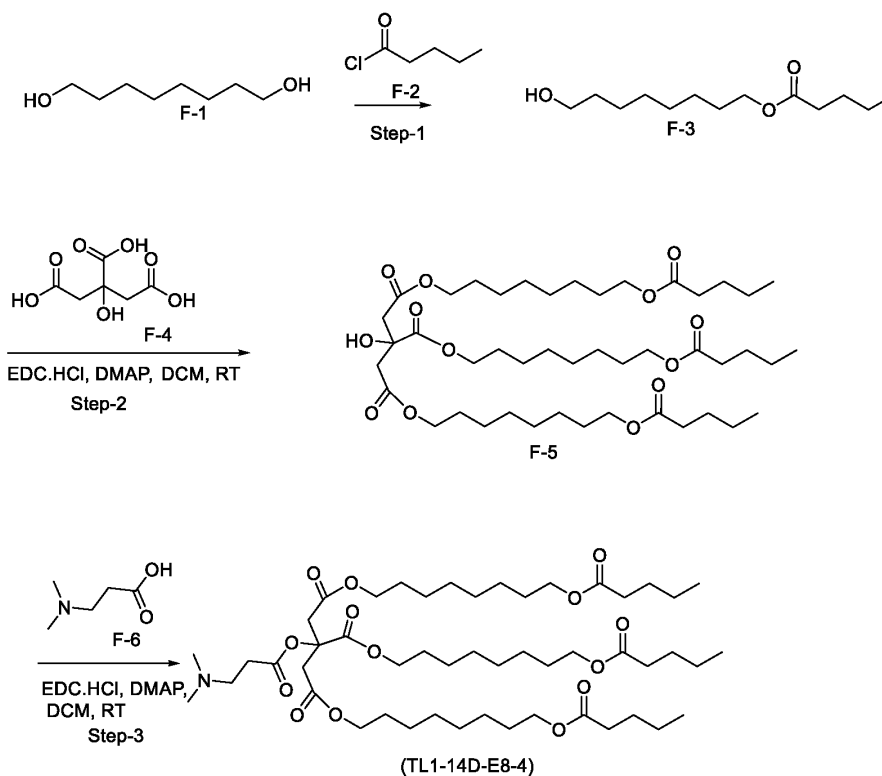
[0607] ¹H NMR (400 MHz, CDCl₃): δ 4.15 (t, *J* = 6.8 Hz, 2H), 4.12-4.03 (m, 10H), 3.29 (d, *J* = 15.6 Hz, 2H), 3.21 (d, *J* = 15.6 Hz, 2H), 2.59-2.56 (m, 2H), 2.49-2.44 (m, 2H), 2.27 (t, *J* = 7.6 Hz, 6H), 2.21 (s, 6H), 1.71-1.60 (m, 18H), 1.44-1.37 (m, 6H), 0.94 (t, *J* = 7.6 Hz, 9H).

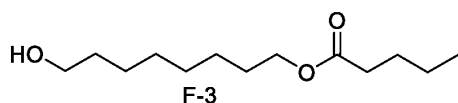
- 10 **[0608]** *ELSD analysis*: Purity 99.79 %, Calculated C₃₈H₆₅NO₁₄ = 759.44, Observed = 760.15 (*m/z*, M+H⁺).

Example 6. Synthetic Protocol for M1266-J03996-029 (TL1-14D-E8-4) (Compound (28))

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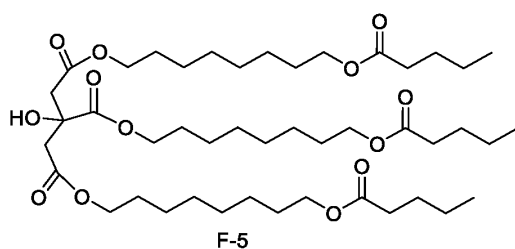
Synthetic Scheme F



Intermediate-F-3

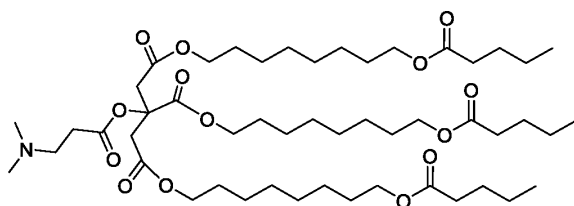
[0609] To a stirred solution of 1, 8-octanediol (10 g, 0.084 mol) and triethylamine (45ml, 0.34 mol) in dichloromethane (200 ml) was cooled to 0°C, then added pentanoyl chloride (4.09 g, 0.034 mol) drop wise. Reaction mass was allowed to RT and stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100 mL) and dried over sodium sulphate. Distilled out solvent under reduced pressure and resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product [F-3] (5.1 g, 32.23%) as yellow liquid.

[0610] ¹H NMR (400 MHz, CDCl₃): δ 4.04 (t, *J* = 6.4 Hz, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.29 (t, *J* = 7.6 Hz, 2H), 1.61 (m, 5H), 1.36 (m, 12H), 0.90 (t, *J* = 6.4 Hz, 3H).

Intermediate-F-5

[0611] To a stirred solution of 8-hydroxyoctyl pentanoate [Int-3] (4.5 g, 0.195 mol) and citric acid (0.937 g, 0.00488 mol) in dichloromethane (20 ml) was added EDCI (3.75 g, 0.0195 mol) followed by addition of DMAP (0.60 g, 0.00488 mol). Reaction mass was stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was diluted with DCM (25.0 mL), washed with DM water (50 ml) and dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using 20 % ethyl acetate in n-heptane to get desired product [F-5] (0.8 g, 19.80%) as yellow colour liquid.

[0612] LCMS analysis: Purity 99 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.25 (*m/z*, M+H⁺).

TL1-14D-E8-4 (Compound (28))

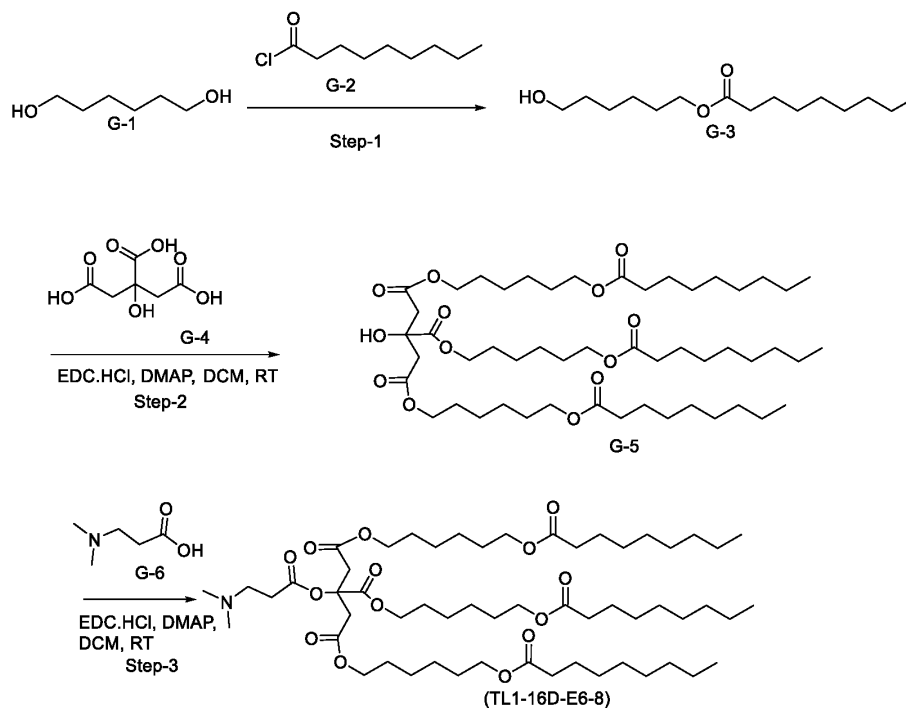
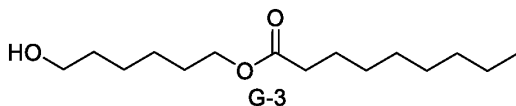
(TL1-14D-E8-4)

Compound (28)

- [0613]** To a stirred solution tris(8-(pentanoyloxy)octyl) 2-hydroxypropane-1,2,3-tricarboxylate
- 5 **[F-5]** (0.8 g, 0.00964 mol) and N,Ndimethylpropionic acid (0.451 g, 0.00385 mol) in dichloromethane (10 ml) was added EDCI (0.740 g, 0.00385 mol,) followed by addition of DMAP (0.117g, 0.000964 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was diluted with DCM (25.0 mL), washed with DM water (50 ml) and dried over sodium sulphate. Distilled out solvent and resulting
- 10 crude was purified over silica using 10% ethyl acetate in n-heptane to obtain desired product TL1-14D-E8-4 (0.30 g, 25%) as yellow colour liquid.

[0614] ¹H NMR (400 MHz, DMSO-d₆): δ 4.13 (t, *J* = 6.4 Hz, 2H), 4.06 (m, 10H), 3.31-3.19 (m, 4H), 2.58 (t, *J* = 7.2 Hz, 2H) 2.48 (t, *J* = 7.2 Hz, 2H) 2.29 (t, *J* = 7.6 Hz, 6H), 2.21 (s, 6H), 1.64-1.56 (m, 17H), 1.38-1.31 (m, 31H), 0.91 (t, *J* = 7.2, 9H).

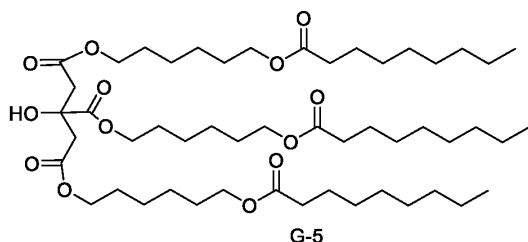
- 15 **[0615]** LCMS analysis: Purity 99.34 %, Calculated C₅₀H₈₉NO₁₄ = 927.63, Observed = 928.25 (m/z, M+H⁺).

Example 7. Synthetic Protocol for M1266-J03996-019 (TL1-16D-E6-8) (Compound (57))**Synthetic Scheme G****Intermediate-G-3**

5

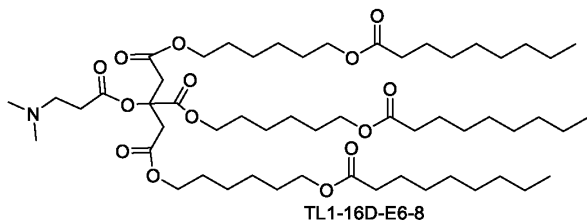
[0616] A stirred solution of 1,6-hexanediol (10 g, 0.084 mol) and trimethylamine (55.92 ml, 0.42 mol) in dichloromethane (200 ml) was cooled to 0°C. Added nonanoyl chloride (7.42 g, 0.042 mol) drop wise. Reaction mass was allowed to stirred at RT for 16 h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100.0 mL), dried over sodium sulphate and concentrated. Resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product **[G-3]** (5.0 g, 22.90%) as yellow liquid.

[0617] ¹H NMR (400 MHz, CDCl₃): δ 4.06 (t, *J* = 6.4 Hz, 2H), 3.64 (m, 2H), 2.28 (t, *J* = 7.6 Hz, 2H), 1.66-1.59 (m, 6H), 1.43-1.20 (m, 14H), 0.89 (t, *J* = 6.4 Hz, 3H).

Intermediate G-5

[0618] To a stirred solution of 6-hydroxyhexyl nonanoate **[G-3]** (4.5 g, 0.0174 mol) and citric acid (1.06 g, 0.0055 mol) in dichloromethane (100 ml) was added EDCI (4.25 g, 0.022 mol) followed by DMAP (0.076 g, 0.0055 mol). Reaction mass was stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100 mL), dried over sodium sulphate and concentrated. Resulting crude was purified over silica using 10% ethyl acetate in n-heptane to obtain desired product **[G-5]** 0.7 g, (13.39%) as yellow colour liquid mass.

[0619] LCMS analysis: Purity 90.81%, Calculated $C_{51}H_{92}O_{13}$ = 912.65, Observed = 913.35 (m/z, $M+H^+$).

TL1-16D-E6-8 (Compound (57))

(Compound (57))

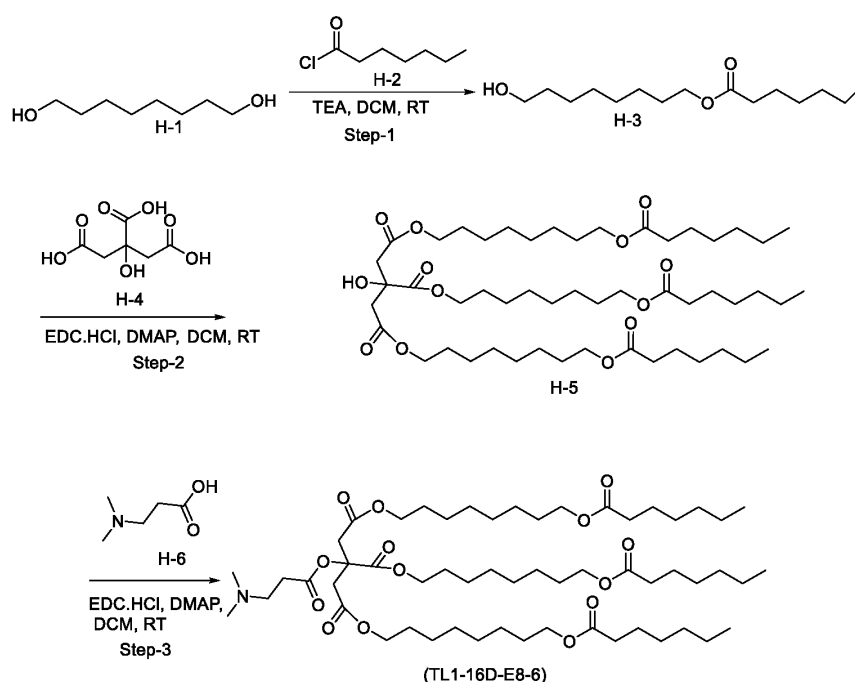
[0620] To a stirred solution of tris(6-(nonanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate **[G-5]** (0.7 g, 0.000766 mol) and N,N-dimethylpropionic acid **[6]** (0.358 g, 0.00306 mol) in dichloromethane (25 ml) was added EDCI (0.590 g, 0.003061 mol,) followed by addition of DMAP (0.11g, 0.00090 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM consumed). Reaction mass was quenched with DM water (100 ml), extracted with DCM (2x 50.0 mL), dried over sodium sulphate and concentrated. Crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product **[TL1-16D-E6-8]** 0.250 g, (32.25%) as yellow colour liquid mass.

[0621] ^1H NMR (400 MHz, DMSO- d_6): δ 4.21 -4.15 (t, J = 6.4 Hz, 2H), 4.08 (m, 10H), 3.31-3.19 (m, 4H), 2.58 (t, J = 7.2 Hz, 2H) 2.48 (m, 2H) 2.29 (t, J = 7.6 Hz, 6H), 2.218 (s, 6H), 1.61 (br, 18H), 1.37 (bs, 12H), 1.28-1.26 (m, 30H) 0.88 (t, J = 7.2, 9H).

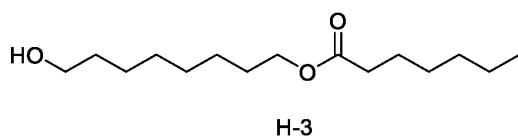
[0622] LCMS analysis: Purity 96.13 %, Calculated $\text{C}_{56}\text{H}_{101}\text{NO}_{14}$ = 1011.72, Observed = 1012.35 (m/z, $\text{M}+\text{H}^+$).

Example 8. Synthetic Protocol for M1266-J03996-031(TL1-16D-E8-6) (Compound (54))

Synthetic Scheme H



10 **Intermediate H-3**



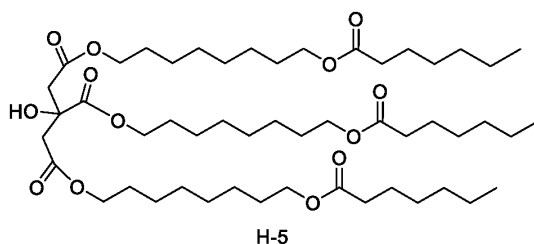
[0623] To a stirred solution of 1,8, octanediol (10 g, 0.084 mol) and triethylamine (45ml, 0.34 mol) in dichloromethane (200 ml) was cooled to 0°C. Added heptenoyl chloride (5.05 g, 0.034 mol) as drop wise. Reaction mass was allowed to RT and stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mixture was washed with DM water 01 L, dichloromethane layer was separated and dried over sodium sulphate. Distilled out solvent under

reduced pressure and resulting crude was purified over silica using 10% ethyl acetate in n-heptane to obtain [H-3] 5.2 g, (29.42%) as yellow colour liquid mass.

[0624] ^1H NMR (400 MHz, CDCl_3): δ 4.04 (t, J = 6.4 Hz, 2H), 3.62 (t, J = 6.4 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.62 (m, 6H), 1.38 (m, 15H), 0.87 (t, J = 6.4 Hz, 3H).

5

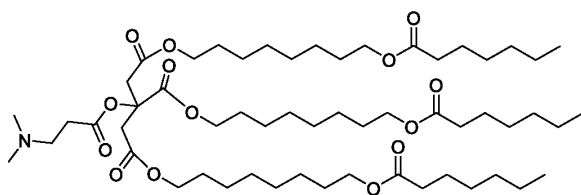
Intermediate H-5



[0625] To a stirred solution of 8-hydroxyoctyl heptanoate [H-3] (5.0 g, 0.143 mol) and citric acid (0.928 g, 0.00483 mol) in dichloromethane (100 ml) was added EDCI (3.71 g, 0.0193 mol) followed by addition of DMAP (0.58 g, 0.0193 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mixture was washed with DM water (50 ml) and dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using 10% ethyl acetate in n-heptane to obtain desired product [Int-5] (0.820 g, 18.63%) as yellow colour liquid mass.

[0626] LCMS analysis: Purity 98.36 %, Calculated $\text{C}_{51}\text{H}_{92}\text{O}_{13}$ = 912.65, Observed = 913.35 (m/z , $\text{M}+\text{H}^+$).

TL1-16D-E8-6 (Compound (54))



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(TL1-16D-E8-6)

(Compound (54))

[0627] To a stirred solution tris(8-(heptanoyloxy)octyl) 2-hydroxypropane-1,2,3-tricarboxylate [H-5] (0.820 g, 0.00897 mol) and N,N-dimethylpropionic acid [Int-6] (0.420 g, 0.00359 mol) in dichloromethane (50 ml), was added EDCI (0.690 g, 0.00359 mol,) followed by addition of DMAP (0.109g, 0.000897 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was

25

monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (50 ml) and dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using 20 % ethyl acetate in n-heptane to obtain desired product [TL1-16D-E8-6] 0.250 g, (27.77%) as yellow colour liquid mass.

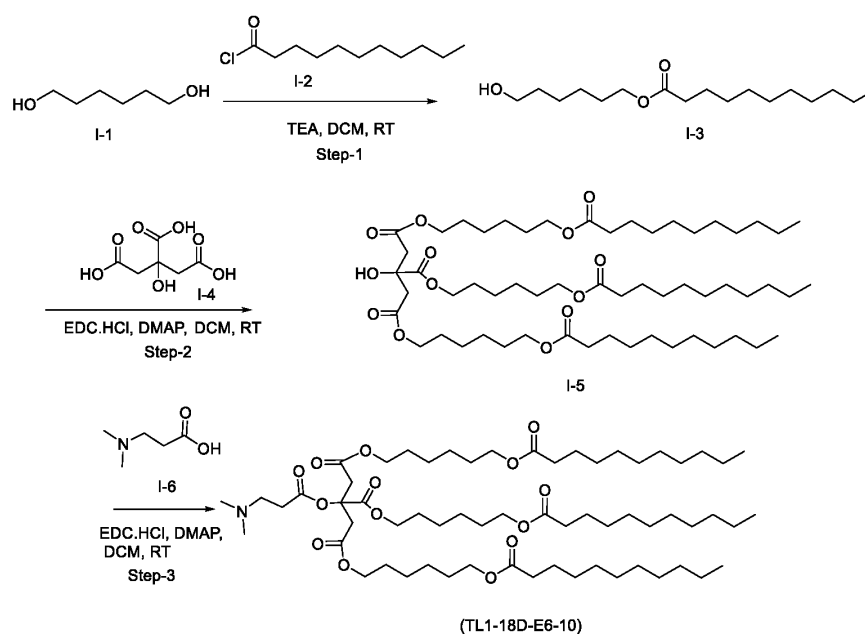
- 5 **[0628]** ^1H NMR (400 MHz, DMSO- d_6): δ 4.127(t, J = 6.4 Hz, 2H), 4.061(m, 10H), 3.310-3.190(m, 4H), 2.583(t, J = 7.2 Hz, 2H) 2.478 (t, J = 7.2 Hz, 2H) 2.84(t, J = 7.6 Hz, 6H), 2.215 (s, 6H), 1.699 (s, 1H), 1.607 (m, 17H), 1.311(m, 44H), 0.877(t, J = 7.2, 9H).

[0629] LCMS analysis: Purity 98.95 %, Calculated $\text{C}_{56}\text{H}_{101}\text{NO}_{14}$ = 1011.72, Observed = 1012.30 (m/z, $\text{M}+\text{H}^+$).

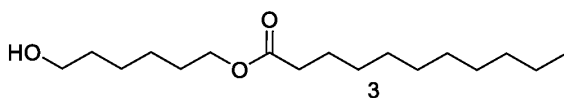
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Example 9. Synthetic Protocol for M1266-J03996-021 (TL1-18D-E6-10) (Compound (56))

Synthetic Scheme I



Intermediate I-3



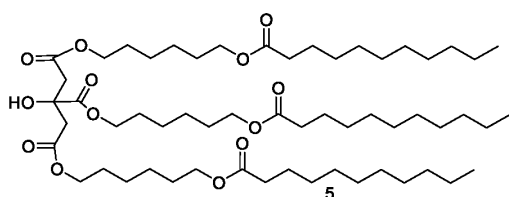
15

[0630] A stirred solution of 1,6-hexanediol (10 g, 0.084 mol) and triethylamine (55.92 ml, 0.42 mol) in dichloromethane (200 ml), was cooled to 0°C and added undecanoyl chloride (8.59 g, 0.042 mol) drop wise. Reaction mass was allowed to stirred at RT for 16 h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100

mL), dried over sodium sulphate. Distilled out solvent under reduced pressure and resulting crude was purified over silica using 10% ethyl acetate in n-heptane. Obtained pure wt 7.3 g of **I-3**, (30.12%) as yellow colour liquid mass.

[0631] ^1H NMR (400 MHz, CDCl_3): δ 4.06 (t, $J=6.4$ Hz, 2H), 3.64 (t, $J=6.4$ Hz, 2H), 2.28 (t, $J=7.6$ Hz, 2H), 1.63 (m, 5H), 1.43 (m, 4H), 1.25 (m, 15H), 0.87 (t, $J=6.4$ Hz, 3H).

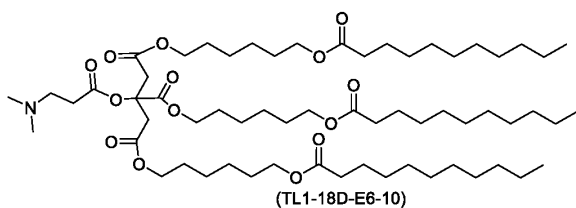
Intermediate I-5



[0632] To a stirred solution of 6-hydroxyhexyl undecanoate [**I-3**] (7.0 g, 0.02443 mol) and citric acid [**4**] (1.48 g, 0.0077 mol) in dichloromethane (100 ml) was added EDCI (5.95 g, 0.0310 mol) followed by addition of DMAP (0.946 g, 0.0077 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mixture was washed with DM water (500 ml), dried over sodium sulphate and concentrated. Crude was purified over silica using 10-15 % ethyl acetate in n-heptane to get desired product [**I-5**] 0.550 g, (7.25%) as yellow liquid mass.

[0633] LCMS analysis: Purity 99.54 %, Calculated $\text{C}_{57}\text{H}_{104}\text{O}_{13}$ = 996.75, Observed = 1019.40 (m/z , $\text{M}+\text{H}^++23$).

TL1-18D-E6-10 (Compound (56))



[0634] To a stirred solution of tris(6-(undecanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [**I-5**] (0.550 g, 0.00055 mol) and N,N-dimethylpropionic acid [**I-6**] (0.258 g, 0.002207 mol) in dichloromethane (25 ml) was added EDCI (0.423 g, 0.002207 mol,) followed by addition of DMAP (0.07g, 0.00055 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was

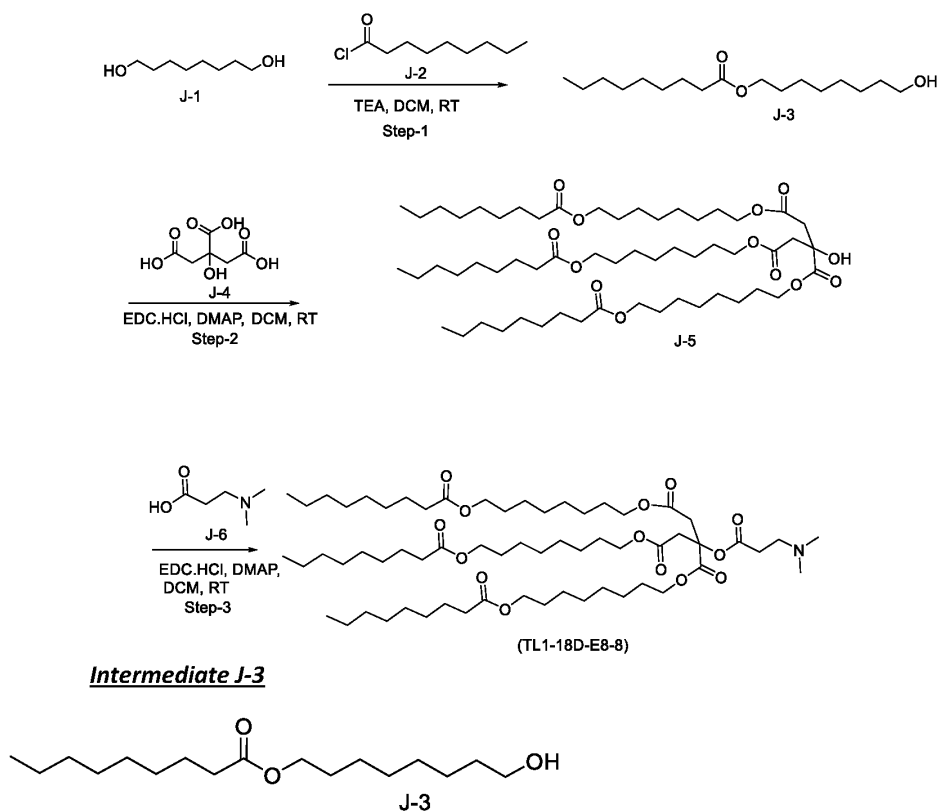
monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (25 ml) and dried over sodium sulphate and concentrated. Crude was purified over silica using 10% ethyl acetate in n-heptane. Obtained pure product, [TL1-18D-E6-10] 0.150 g, (24.83%) as yellow liquid.

[0635] ¹H NMR (400 MHz, CDCl₃): δ 4.14 (t, *J* = 6.4 Hz, 2H), 4.08 (m, 10H), 3.31-3.19 (m, 4H),
 5 2.59 (t, *J* = 7.2 Hz, 2H) 2.48 (t, *J* = 7.2 Hz, 2H) 2.28 (t, *J* = 7.6 Hz, 6H), 2.22 (s, 6H), 1.61 (m, 18H),
 1.37 (br, 12H), 1.28 (m, 42H) 0.87 (t, *J* = 7.2, 9H).

[0636] LCMS analysis: Purity 99.87 %, Calculated C₆₂H₁₁₃NO₁₄ = 1095.82, Observed = 1096.40 (m/z, M+H⁺).

10 Example 10. Synthetic Protocol for TL1-18D-E8-8 (Compound (53))

Synthetic Scheme J



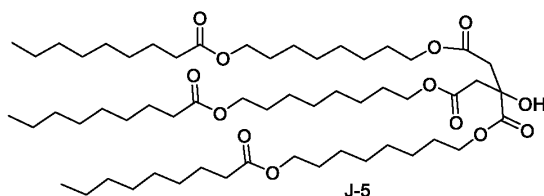
15

[0637] A stirred solution of octane diol (10 g, 0.068 mol) in dichloromethane (200 ml) was cooled to 0°C and then added triethylamine (45.26 ml, 0.34 mol). Reaction mass was stirred for 05 minute then added DMAP (4.17 g, 0.034 mol) followed by nonanoyl chloride (6.0 g, 0.034 mol)

drop wise. Resulting reaction mass was allowed to stirred at RT for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100 mL), dried over sodium sulphate and concentrated. Resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get **[J-3]** (5.2 g, 26.55%) as yellow colour liquid mass.

- 5 **[0638]** ¹H NMR (400 MHz, CDCl₃): δ 4.04 (t, J = 6.4 Hz, 2H), 3.65 (m, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.57 (m, 4H), 1.280 (m, 20H), 0.888 (t, J = 6.4 Hz, 3H).

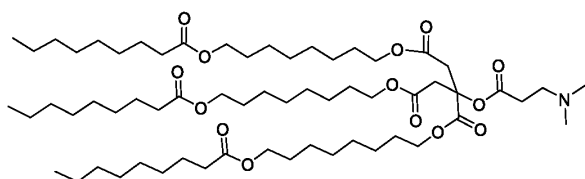
Intermediate J-5



- 10 **[0639]** To a stirred solution of 8-hydroxyoctyl nonanoate **[J-3]** (5.0 g, 0.0174 mol) and citric acid (1.06 g, 0.0055 mol) in dichloromethane (100 ml) was added EDCI (4.25 g, 0.022 mol) followed by addition of DMAP (0.076 g, 0.0055 mol). Reaction mass was stirred for 16 h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was diluted with DCM (100.0 mL) and washed with DM water (100.0 mL), dried over sodium sulphate and concentrated.
- 15 Resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get **[I-5]** (0.9 g, 15.7%) as yellow colour liquid mass.

[0640] ¹H NMR (400 MHz, DMSO-d₆): δ 6.92 (s, 1H), 4.20-4.15 (m, 4H), 4.11 (m, 8H), 4.01 (s, 2H), 3.95 (s, 2H), 2.28 (t, J = 7.6 Hz, 6H), 1.63 (m, 18H), 1.33 (m, 52H) 0.83 (m, 9H).

- 20 **TL1-18D-E8-8 (Compound (53))**



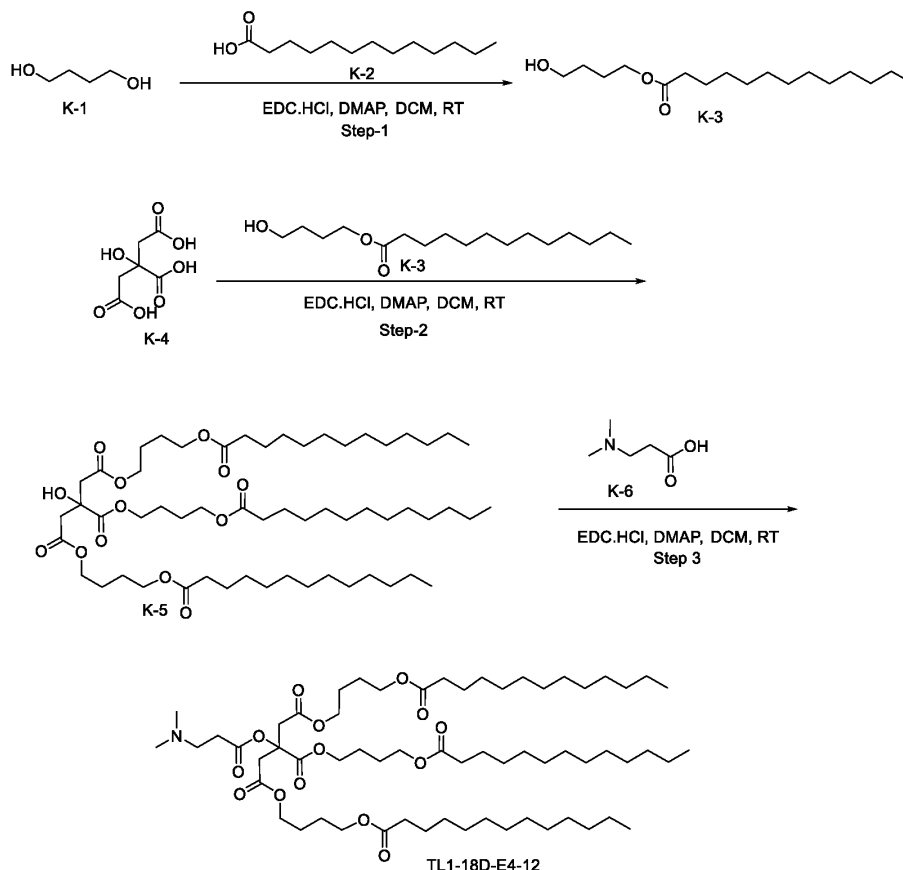
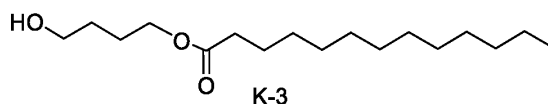
(Compound (53))

[0641] To a stirred solution of tris(8-(nonanoyloxy)octyl) 2-hydroxypropane-1,2,3-tricarboxylate **[I-5]** (0.9 g, 0.00090 mol) and N,N-dimethylpropionic acid (0.422 g, 0.0036 mol) in

dichloromethane (10 ml) was added EDCI (0.7 g, 0.00361 mol,) followed by addition of DMAP (0.11g, 0.00090 mol). Reaction mass was stirred for 16 h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was diluted with (50.0 mL) and washed with DM water (100 ml) dried over sodium sulphate and concentrated. Resulting crude
5 was purified over silica using 10% ethyl acetate in n-heptane to get final [TL1-18D-E8-8] (0.288 g, 29.12%) as yellow colour liquid mass.

[0642] ^1H NMR (400 MHz, CDCl_3): δ 4.11 (t, J = 6.8 Hz, 2H), 4.01 (m, 10H), 3.31-3.27 (d, J = 15.6 Hz, 2H), 3.23-3.19 (d, J = 15.6 Hz, 2H), 2.60-2.56 (m, 2H), 2.49-2.46 (m, 2H), 2.28 (t, J = 7.6 Hz, 6H), 2.21 (s, 6H), 1.66-1.57 (m, 18H), 1.31-1.26 (m, 54H), 1.24 (brs, 28H), 0.87 (t, J = 6.8 Hz, 9H).

10 **[0643]** *LCMS analysis*: Purity 99.37 %, Calculated $\text{C}_{62}\text{H}_{113}\text{NO}_{14}$ = 1095.82, Observed = 1096.40 (m/z, $\text{M}+\text{H}^+$).

Example 11. Synthetic Protocol for M1266-J03983-035 (JC-TL1-18D-E4-12) (Compound (22))**Synthetic Scheme K****Intermediate [K-3]:**

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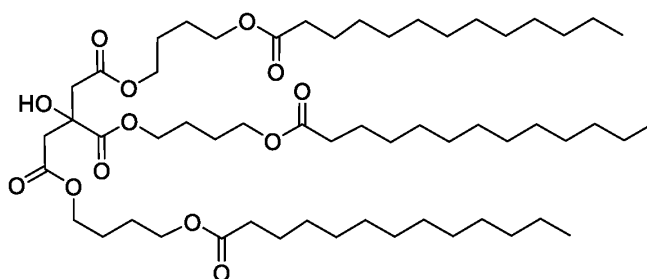
[0644] To a stirred solution of butane-1,4-diol **[K-1]** (10 g, 0.111 mol) and tridecanoic acid **[K-2]** (23.7 g, 0.111 mol) in dichloromethane (300 mL) was cooled to 0 °C, EDC.HCl (25.5 g, 0.133 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (6.7 g, 0.055 mol) and stirred for 16 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-20 % ethyl acetate in n-

hexane), to obtain pure product 4-hydroxybutyl tridecanoate **[K-3]** (11.8 g, 35 % Yield) as a pale yellow liquid.

[0645] ^1H NMR (400 MHz, CDCl_3): δ 4.10 (t, J = 6.4 Hz, 2H), 3.71-3.66 (m, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.76-1.57 (m, 6H), 1.27-1.11 (m, 18H), 0.89-0.85 (m, 3H).

5

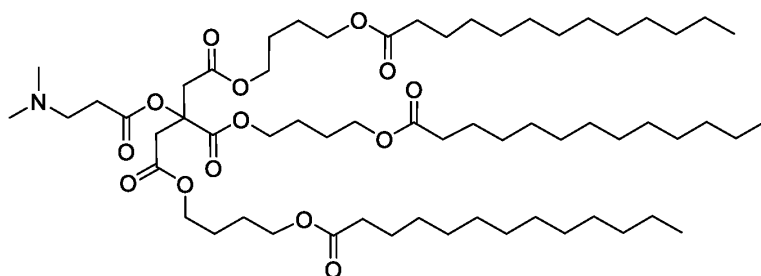
Intermediate [K-5]:



- [0646]** To a stirred solution of 4-hydroxybutyl tridecanoate **[K-3]** (11.8 g, 0.04123 mol) and Citric acid **[K-4]** (2.0 g, 0.0103 mol) in dichloromethane (150 mL) was cooled to 0 °C, EDC.HCl (7.90 g, 0.0412 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (1.25 g, 0.0103 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product tris(4-(tridecanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate **[K-5]** (1.1 g, 10 % Yield) as a pale yellow liquid.

[0647] *ELSD analysis:* Purity 99.74 %, Calculated $\text{C}_{57}\text{H}_{104}\text{O}_{13}$ = 996.75, Observed = 1019.30 (m/z , $\text{M}+\text{Na}^+$).

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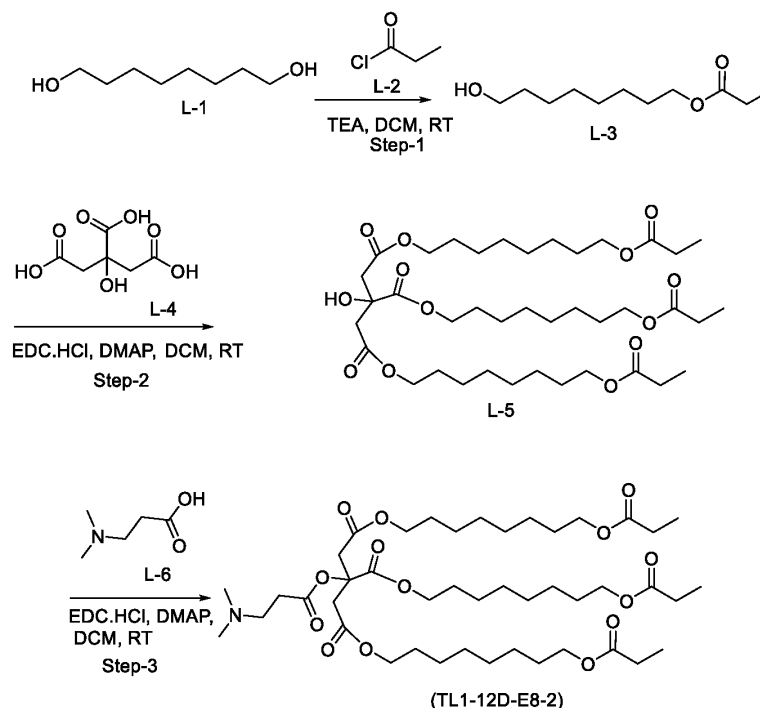
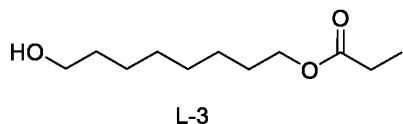
JC-TL1-18D-E4-12 (Compound (22))

(Compound (22))

[0648] To a stirred solution of tris(4-(tridecanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate **[K-5]** (1.1 g, 0.0011 mol) and 3-(dimethylamino)propanoic acid **[K-6]** (0.51 g, 0.0044 mol) in dichloromethane (20 mL) was cooled to 0 °C, EDC.HCl (0.84 g, 0.0044 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.134 g, 0.0011 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (10 mL) was added to the reaction mixture and extracted with DCM (3x25 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product tris(4-(tridecanoyloxy)butyl) 2-((3-(dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate **[JC-TL1-18D-E4-12]** (Compound (22)) (0.2 g, 16 %, Yield) as a colourless liquid.

[0649] ¹H NMR (400 MHz, CDCl₃): δ 4.19-4.16 (m, 2H), 4.10-4.08 (m, 10H), 3.30 (d, *J* = 15.6Hz, 2H), 3.23 (d, *J* = 15.6Hz, 2H), 2.59-2.56 (m, 2H), 2.49-2.46 (m, 2H), 2.28 (t, *J* = 7.6Hz, 6H), 2.21 (s, 6H), 1.70-1.69 (m, 12H), 1.62-1.59 (m, 7H), 1.31-1.28 (m, 53H), 0.87 (t, *J* = 6.8Hz, 9H).

[0650] *ELSD analysis*: Purity 99.91 %, Calculated C₆₂H₁₁₃NO₁₄ = 1095.82, Observed = 1096.45 (*m/z*, M+H⁺).

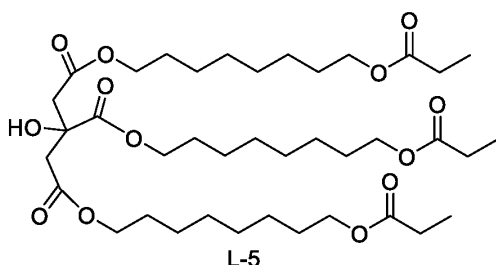
Example 12. Synthetic Protocol for M1266-J03996-027 (TL1-12D-E8-2) (Compound (11))**Synthetic Scheme L****Intermediate L-3**

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[0651] To a stirred solution of 1, 8-octanediol (10 g, 0.084 mol) and triethylamine (45ml, 0.34 mol) in dichloromethane (200 ml) was cooled to 0°C, then added propionoyl chloride (3.14 g, 0.034 mol) drop wise. Reaction mass was allowed to stirred at RT for 16 h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mixture was washed with DM water (100.0 mL), dried over sodium sulphate and concentrated under reduced pressure. Crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product **[L-3]** (7.3 g; 30.12%) as yellow liquid.

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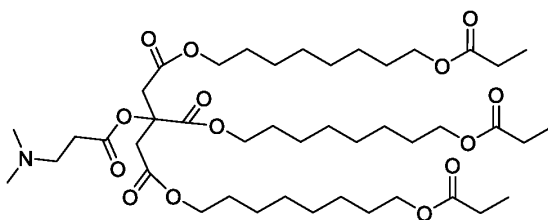
[0652] ¹H NMR (400 MHz, CDCl₃): δ 4.05 (t, *J* = 6.4 Hz, 2H), 3.62 (q, *J* = 6.0 Hz, 2H), 2.30 (q, *J* = 7.6 Hz, 2H), 1.61 (m, 3H), 1.35 (s, 9H), 1.11 (t, *J* = 6.4 Hz, 3H).

Intermediate L-5

[0653] To a stirred solution of 8-hydroxyoctyl propionate [L-3] (5.3 g, 0.062 mol) and citric acid (1.25 g, 0.00655 mol) in dichloromethane (20 ml) was added EDCI (5.03 g, 0.0262 mol) followed by addition of DMAP (0.80 g, 0.00635 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (Sm was consumed). Reaction mass was washed with DM water (50 ml), dried over sodium sulphate and concentrated. Crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product [Int-5] (1.1 g, 22.68%) as yellow liquid.

[0654] ¹H NMR (400 MHz, CDCl₃): δ 4.059(t, *J* = 6.8 Hz, 6H), 3.643(d, *J* = 3.2 Hz, 6H), 2.282(t, *J* = 7.6 Hz, 6H), 1.629(m, 18H), 1.331 (m, 22H) 0.829(m, 9H).

[0655] LCMS analysis: Purity 98.35 %, Calculated C₃₉H₆₈O₁₃ = 744.47, Observed = 745.20 (*m/z*, M+H⁺).

TL1-12D-E8-2 (Compound (11))

(TL1-12D-E8-2)

(Compound (11))

[0656] To a stirred solution of tris(8-(propionyloxy)octyl) 2-hydroxypropane-1,2,3-tricarboxylate [L-5] (1.1 g, 0.00147 mol) and N,Ndimethylpropionic acid (0.691 g, 0.00590 mol) in dichloromethane (10 ml) was added EDCI (1.13 g, 0.005906 mol,) followed by addition of DMAP (0.180g, 0.00147 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water 20 ml, dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using

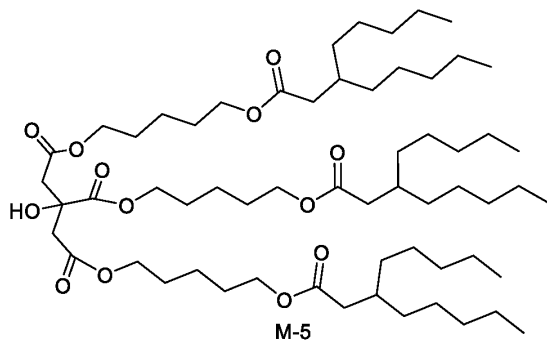
15 **[0659]** To a stirred solution of 1,5, pentanediol (20 g, 0.190 mol) and triethylamine (90ml, 0.68 mol) in dichloromethane (200 ml) was cooled to 0°C. Added 3-pentyl octanoyl chloride (22.13 g,

0.095 mol) drop wise. Reaction mass was allowed to RT and stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (100 mL), dried over sodium sulphate and concentrated under reduced pressure. Resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product **[M-3]** (17.0 g, 60.71%) as yellow colour liquid.

[0660] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.4 Hz, 2H), 3.66 (t, J = 6.4 Hz, 2H), 2.23 (d, J = 6.8 Hz, 2H), 1.83 (s, 1H), 1.69-1.58 (m, 4H), 1.47-1.41 (m, 2H), 1.25 (s, 17H), 0.88 (t, J = 6.4 Hz, 6H).

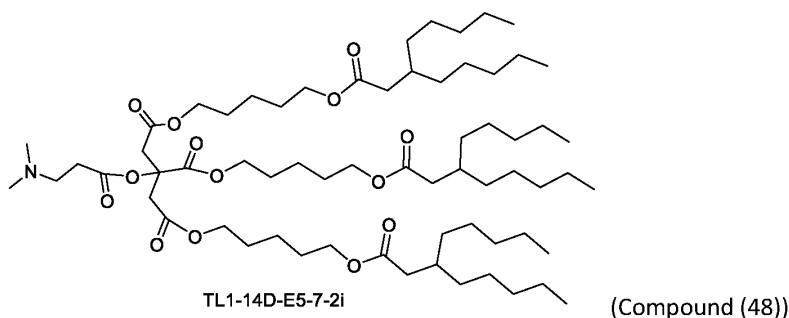
[0661] LCMS analysis: Purity 99.96 %, Calculated $\text{C}_{18}\text{H}_{36}\text{O}_3$ = 300.27, Observed = 301.30 (m/z , $\text{M}+\text{H}^+$).

Intermediate M-5



[0662] To a stirred solution of 5-hydroxypentyl 3-pentyloctanoate **[M-3]** (17 g, 0.056 mol) and citric acid (2.72 g, 0.0141 mol) in dichloromethane (60 ml) was added EDCI (10.82 g, 0.056 mol) followed by DMAP (1.72 g, 0.0141 mol). Reaction mass was stirred for 16h. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mass was washed with DM water (50 ml), dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using 10% ethyl acetate in n-heptane to get desired product **[Int-5]** (12 g, 85%) as yellow colour liquid mass.

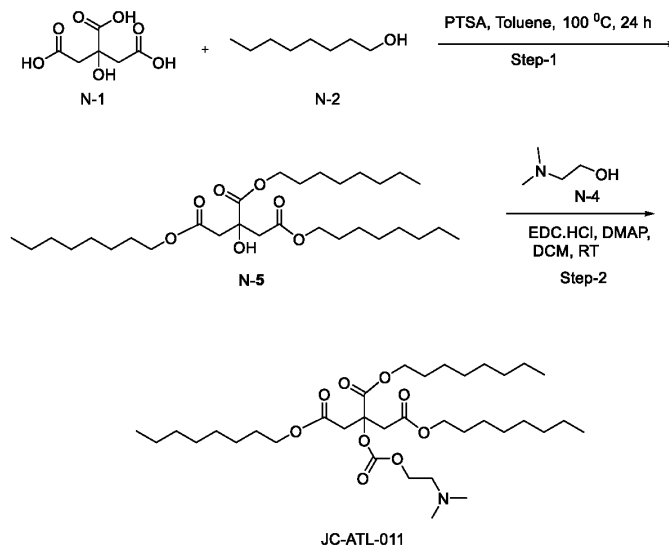
[0663] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.8 Hz, 2H), 4.05 (m, 10H), 2.89-2.77 (m, 4H), 2.23-2.21 (d, J = 7.2 Hz, 6H), 1.83 (m, 3H), 1.72-1.61 (m, 12H) 1.56 (m, 6H), 1.39 (m, 8H), 1.32-1.26 (m, 40H), 0.85 (m, 18H).

TL1-14D-E5-7-2i (Compound (48))

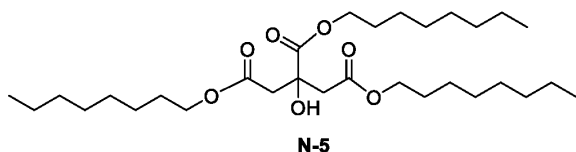
- [0664]** To a stirred solution of tris(5-((3-pentyloctanoyl) oxy) pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [Int-5] (2.5 g, 0.00240 mol) and N,N-dimethyl propionic acid [Int-6] (1.12 g, 0.00962 mol) in dichloromethane (100 ml) was added EDCI (2.0 g, 0.00962 mol,) followed by addition of DMAP (0.30 g, 0.0024 mol). Reaction mass was stirred for 16h at RT. Progress of reaction was monitored by ELSD/TLC (SM was consumed). Reaction mixture was washed with DM water (50 ml) and dried over sodium sulphate. Distilled out solvent and resulting crude was purified over silica using 30% ethyl acetate in n-heptane to get desired product [TL1-14D-E5-7-2i] 1.2 g, (44.44%) as yellow colour liquid mass.

[0665] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, $J = 6.4$ Hz, 2H), 4.08 (m, 10H), 3.31-3.19 (m, 4H), 2.59 (t, $J = 7.2$ Hz, 2H), 2.47 (t, $J = 7.2$ Hz, 2H), 2.22-2.21 (m, 12H), 1.83 (m, 3H), 1.69-1.60 (m, 12H), 1.42 (m, 6H), 1.30-1.22 (m, 48H), 0.87 (t, $J = 7.2$, 18H).

- [0666]** LCMS analysis: Purity 99.83 %, Calculated $\text{C}_{65}\text{H}_{119}\text{NO}_{14} = 1137.86$, Observed = 1138.45 (m/z, $\text{M}+\text{H}^+$).

Example 14. Synthetic Protocol for M1266-450487 (JC-ATL-011) (Compound (6))**Synthetic Scheme N****Intermediate N-5**

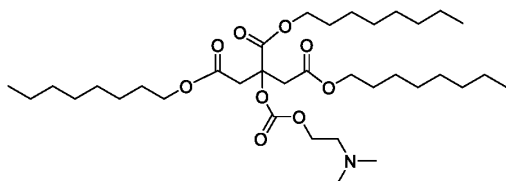
5



[0667] To a solution of citric acid (25.0 g, 0.130 mol) and octan-1-ol (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol,) was added, and the resulting mixture was stirred at 100 °C for 24h. The reaction progress was monitored by TLC (5% EtOAc/hexanes). The reaction mixture was cooled to r.t, diluted with water (500 mL) and extracted with ethyl acetate (500 mL x 3). The organic layer was dried over anhydrous Na₂SO₄ and solvent was evaporated. The crude was purified by column chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give compound **[N-5]** (40.0 g, 58.22 %) as a colourless liquid

[0668] ¹H NMR (400 MHz, DMSD-d₆): δ 5.61 (s, 1H), 4.01 (t, *J* = 6.8 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 4H), 2.87 (d, *J* = 15.2 Hz, 2H), 2.72 (d, *J* = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, *J* = 6.4 Hz, 9H).

[0669] LCMS analysis: Purity 97.38%, Calculated C₃₀H₅₆O₇ = 528.40, Observed = 529.2 (*m/z*, M+H⁺).

JC-ATL-011 (Compound (6))

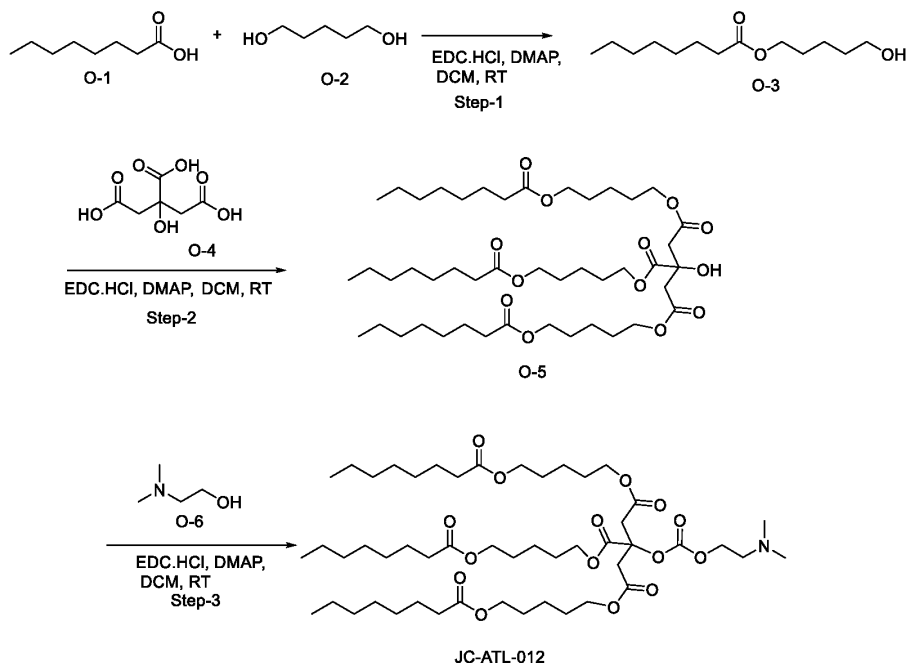
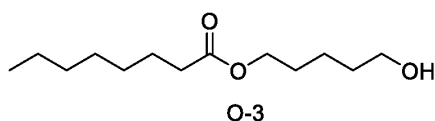
ATL-011

(Compound (6))

[0670] To a stirred solution of 1,2,3-trioctyl 2-hydroxypropane-1,2,3-tricarboxylate **[N-5]** (0.5 g, 946 μ mol) in dichloromethane (30 mL, 469 mmol), was added triethylamine (287 mg, 3 eq., 2.84 mmol) and 4-(dimethylamino)pyridin-1-ium (116 mg, 946 μ mol) at 0 °C. Reaction mass was stirred for 10 minute at same temperature, followed by addition of ditrichloromethyl carbonate (281 mg, 946 μ mol) and 2-(dimethylamino)ethan-1-ol (84.3 mg, 946 μ mol), the resulting reaction mixture was stirred at room temperature for 16 hrs. The progress of reaction was monitor by TLC. The resulting reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (3x30 mL). Whole organic was combined and dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by column chromatography by using 0-5% MeOH in DCM as eluent. The pure fraction was combined and concentrated under reduced pressure to obtain [JC-ATL-011] (Compound (6)) (410 mg, yield-67.37%) as a yellow liquid.

[0671] ¹H NMR (400 MHz, CDCl₃): δ 4.20 (t, *J* = 6.0 Hz, 2H), 4.16-4.14 (d, *J* = 6.8 Hz, 2H), 3.35-3.31 (d, *J* = 15.6 Hz, 2H), 3.24-3.20 (d, *J* = 15.6 Hz, 2H), 2.58 (d, *J* = 6 Hz, 2H), 2.27 (s, 6H), 1.67-1.56 (m, 6H), 1.27 (brs, 30H), 0.87 (t, *J* = 6.4 Hz, 9H).

[0672] LCMS analysis: Purity 99.87 %, Calculated C₃₅H₆₅NO₉ = 644.47, Observed = 644.75 (m/z, M+H⁺).

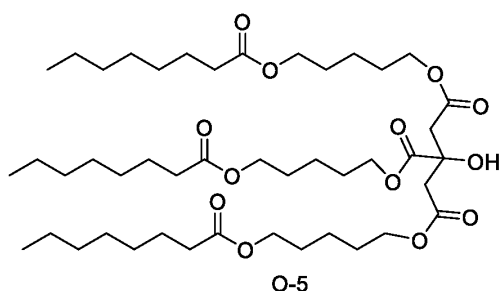
Example 15. Synthetic Protocol for M1266-449313 (JC-ATL-012) (Compound (25))**Synthetic Scheme O****Intermediate O-3**

5

[0673] To a stirred solution of pentane-1,5-diol (6.5 g, 0.9 eq., 62.4 mmol) in dichloromethane (50 mL, 781 mmol) was added octanoic acid (10 g, 69.3 mmol). Reaction mass was cooled to 0°C. Added ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (16.7 g, 1.3 eq., 87.4 mmol), and 4-(dimethylamino)pyridin-1-ium (1.71 g, 0.2 eq., 13.9 mmol). The reaction mixture was stirred at r.t. for 48h. Reaction progress was monitored by TLC / ELSD, after consumption of starting material, the reaction mixture was washed with saturated NaHCO₃ and brine solution. After evaporation of organic layer, resulting crude was purified by flash column chromatography to get compound **O-3** (6.0 g; yield: 37.56 %) as colourless liquid.

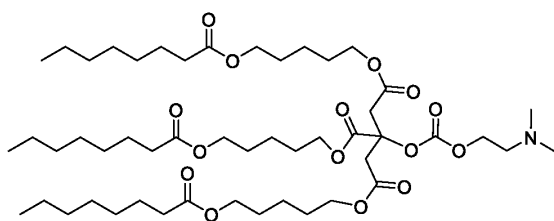
[0674] ¹H NMR (400 MHz, CDCl₃): δ 4.08 (t, *J* = 6.4 Hz, 2H), 3.67 (s, 2H), 2.30 (t, *J* = 7.2 Hz, 2H), 1.71-1.58 (m, 6H), 1.44 (m, 2H), 1.29 (s, 10H), 0.89 (t, *J* = 6.8 Hz, 3H).

15

Intermediate O-5

[0675] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [compound O-3] (250 mg, 1.3 mmol) in dichloromethane (5 mL, 78.1 mmol), was added N,N-dimethylpyridin-4-amine (159 mg, 1.3 mmol) and {[3-(dimethylamino)propyl]imino}methylidene(ethyl)amine hydrochloride (998 mg, 4 eq., 5.2 mmol). Reaction mass stirred 10 min at 25°C, then added 5-hydroxypentyl octanoate (1.35 g; 5.86 mmol). Reaction mixture was stirred for 16 hr at r.t. under nitrogen atmosphere. Reaction progress monitored by TLC / ELSD, after consumption of starting material reaction mass diluted with water and dichloromethane. Organic layer was separated, dried over sodium sulphate. Solvent was distilled out under reduced pressure. Crude was purified with column chromatography using 15-20% ethyl acetate, to get compound O-5 (0.9 g; yield: 83.42 %) as a light yellow colour less liquid.

[0676] ^1H NMR (400 MHz, CDCl_3): δ 4.21 (m, 2H), 4.08 (m, 10H), 2.90-2.77 (dd, J = 15.6 Hz, 4H), 2.29 (t, J = 7.6 Hz, 6H), 1.61(m, 12H), 1.41 (m, 8H), 1.28(brs, 28H), 0.85 (t, J = 6.4 Hz, 9H).

JC-ATL-012 (Compound (25))

JC-ATL-012

(Compound (25))

[0677] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [compound O-5] (0.5 g, 603 μmol) in dichloromethane (6 mL, 93.7 mmol), triethylamine (183 mg, 3 eq., 1.81 mmol) and 4-(dimethylamino)pyridin-1-ium (74.3 mg, 603 μmol) was successively added at 0 °C and stirred for 10 minute at same temperature, followed by

addition of ditrichloromethyl carbonate (179 mg, 603 μmol) and 2-(dimethylamino)ethan-1-ol (53.8 mg, 603 μmol) was added. The resulting reaction mixture was stirred at room temperature for 16 hrs and progress of reaction was monitored by TLC / ELSD. The resulting reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (3x30 mL). Combined
 5 organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was purified by column chromatography by using 0-5% MeOH in DCM as eluent. The pure fraction was combined and concentrated under reduced pressure to obtain [JC-ATL-012] (Compound (25)) (225 mg. Yield 39.51%) as a yellow liquid.

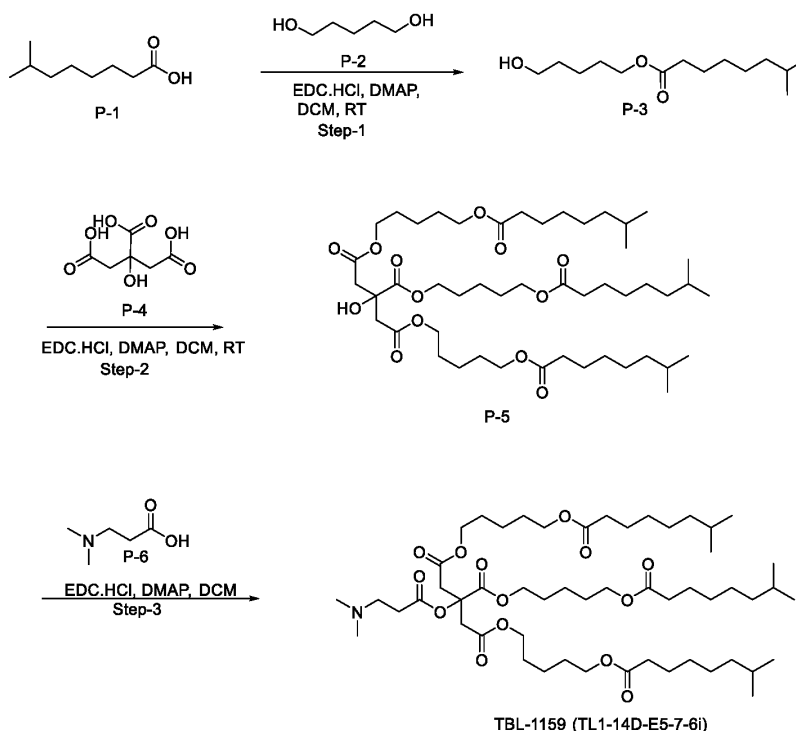
[0678] ^1H NMR (400 MHz, CDCl_3): δ 4.195 (m, 4H), 4.08 (m, 10H), 3.34-3.30 (d, $J = 15.6$ Hz, 2H),
 10 3.24-3.20 (d, $J = 15.6$ Hz, 2H), 2.57 (t, $J = 5.6$ Hz, 2H), 2.28 (t, $J = 8.8$ Hz, 12H), 1.72-1.57 (m, 19H), 1.44 (m, 6H), 1.28 (brs, 24H), 0.87 (t, $J = 6.4$ Hz, 9H).

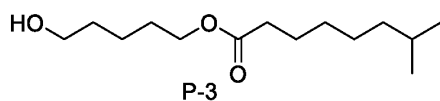
LCMS analysis: Purity 99.74 %, Calculated $\text{C}_{50}\text{H}_{89}\text{NO}_{15} = 943.62$, Observed = 944.60 (m/z , $\text{M}+\text{H}^+$).

Example 16. Synthetic Protocol for M1266-674154 (TL1-14D-E5-7-6i) (Compound (36))

15

Synthetic Scheme P

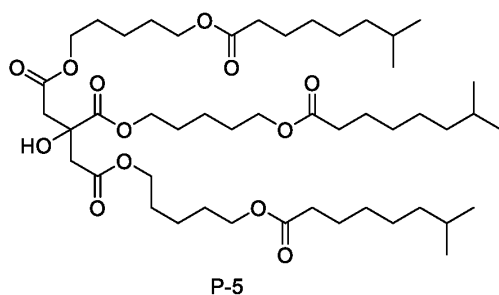


Intermediate P-3

[0679] To the stirred solution of 7-methyloctanoic acid (12 g, 75.8 mmol) in dichloromethane (0.2 L), N,N-dimethylpyridin-4-amine (9.26 g, 75.8 mmol) and ([3-

- 5 (dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (29.1 g, 2 eq., 152 mmol) were added slowly at 0 °C, after 10 min pentane-1,5-diol (15.8 g, 2 eq., 152 mmol) was added. Reaction mixture was allowed to stirred at RT for 18 h. Progress of reaction was monitored by ELSD / TLC. Reaction mixture was diluted with cold water (500.0 mL) and extracted with dichloromethane (3x 100 ml). Combined organic layer was washed with fresh water (2x 100 mL)
- 10 and brine solution (2x 100.0 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude was purified with column chromatography using 10% Ethyl acetate in Hexanes, to give 5-hydroxypentyl 7-methyloctanoate [Int-3] (12 g, 49.1 mmol) as colourless clear liquid.

[0680] ¹H NMR (400 MHz, CDCl₃): δ 4.07 (t, *J* = 6.8 Hz, 2H), 3.68-3.63 (dd, *J* = 5.2 Hz, 2H), 2.29 (t, *J* = 7.6 Hz, 2H), 1.64 (m, 5H), 1.58- 1.41(m, 3H), 1.29 (m, 5H), 1.16(m, 2H), 0.851 (d, *J* = 6.8 Hz, 6H).

Intermediate P-5

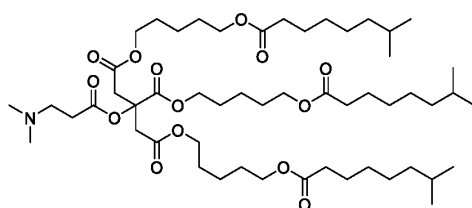
- 20 **[0681]** The solution of 2-hydroxypropane-1,2,3-tricarboxylic acid (2.44 g, 12.7 mmol) in dichloromethane (0.1 L) was cooled to 0°C, then added N,N-dimethylpyridin-4-amine (1.55 g, 12.7 mmol) and ([3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (9.73 g, 4 eq., 50.7 mmol) slowly at room temperature, after 10 min, 5-hydroxypentyl 7-methyloctanoate **[P-3]** (12.4 g, 4 eq., 50.7 mmol) was added drop wise. Reaction mixture was allowed to stirred at
- 25 25°C for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with cold water (100.0 mL) and extracted with dichloromethane (3x 100 mL). Combined organic layer was

washed with fresh water (2x 50 mL) and brine solution (2x 50.0 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude was purified with column chromatography using 10% Ethyl acetate in Hexanes, to give 1,2,3-tris({5-[(7-methyloctanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[P-5]** (4.5 g, 5.17 mmol) as colourless clear liquid.

[0682] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 5.6 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.29 (t, J = 7.6 Hz, 6H), 1.64 (m, 16H), 1.50(m, 4H), 1.49 (m, 6H), 1.28(m, 13H), 1.15 (m, 6H), 0.85 (d, J = 6.8 Hz, 18H).

10

TL1-14D-E5-7-6i (Compound (36))



TBL-1159 (TL1-14D-E5-7-6i)

(Compound (36))

[0683] A stirred solution of 1,2,3-tris({5-[(7-methyloctanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[P-5]** (5.2 g, 5.97 mmol) in dichloromethane (61.2 mL, 956 mmol) was cooled to 0°C. 3-(dimethylamino)propanoic acid (2.8 g, 4 eq., 23.9 mmol) was added and stirred for 10 minute. {3-[cyano(ethyl)amino]propyl}dimethylazanium chloride (4.58 g, 4 eq., 23.9 mmol) was added at same temperature, followed by N,N-dimethylpyridin-4-amine (729 mg, 5.97 mmol). Reaction mass allowed to stirred at room temperature for 16 h. Progress of reaction was monitored by ELSD / TLC. Reaction mixture was diluted with cold water (500.0 mL) and extracted with dichloromethane (2x 150 ml). Combined organic layer was washed with fresh water (2x 100 mL) and brine solution (2x 100.0 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude was purified by column chromatography using 50% Ethyl acetate in Hexanes, to give [TL1-14D-E5-7-6i] (Compound (36)) (1.85 g, 1.91 mmol) as brown colour liquid mass.

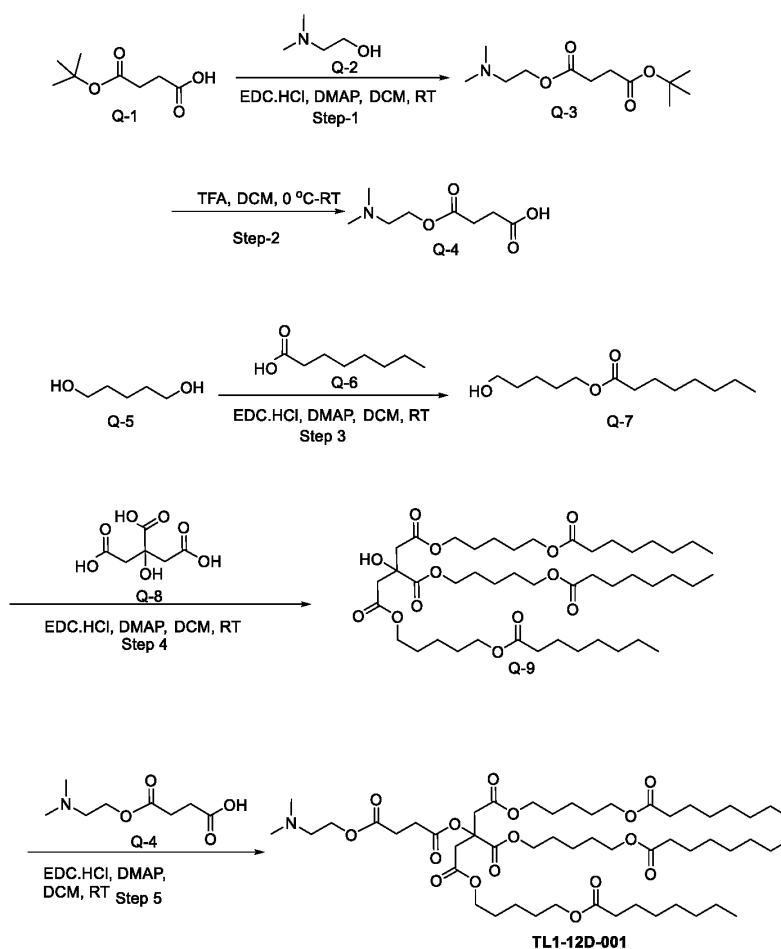
[0684] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, J = 6.8 Hz, 2H), 4.08 (m, 10H), 3.31-3.27 (d, J = 15.6 Hz, 2H), 3.23-3.19 (d, J = 15.6 Hz, 2H), 2.58 (t, J = 7.2 Hz, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.28 (t, J = 7.6 Hz, 6H), 2.22 (s, 6H), 1.69-1.5(m, 18H), 1.51 (m, 3H), 1.42 (m, 6H), 1.29 (m, 12H), 1.14 (m, 6H), 0.87 (d, J = 6.8 Hz, 18H).

[0685] LCMS analysis: Purity 98.45 %, Calculated $C_{53}H_{95}NO_{14}$ = 969.68, Observed = 970.68 (m/z , $M+H^+$).

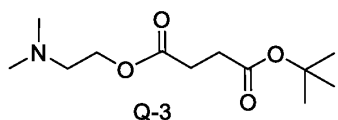
Example 17. Synthetic Protocol for M1266-480517 (TL1-12D-001) (Compound (33))

5

Synthetic Scheme Q



Intermediate [Q-3]:



[0686] To a stirred solution of 4-(tert-butoxy)-4-oxobutanoic acid [**Q-1**] (10 g, 0.0574 mol) and 2-(dimethylamino)ethan-1-ol [**Q-2**] (6.14 g, 0.0689 mol) in dichloromethane (100 mL) was cooled to 0 °C, {[3-(dimethylamino)propyl]imino}methylidene(ethyl)amine hydrochloride (13.9 g, 0.0723 mol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.41 g, 0.0115 mol) and the

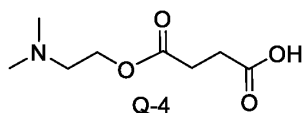
10

reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in

- 5 Dichloromethane), to give the desired tert-butyl (2-(dimethylamino)ethyl) succinate **[Q-3]** (9.0 g, 63.91% yield) as yellow liquid.

[0687] ¹H-NMR (400 MHz, CDCl₃)- δ 4.16 (t, *J* = 6.0 Hz, 2H), 2.59-2.41 (m, 6H), 2.24 (s, 6H), 1.41 (s, 9H)

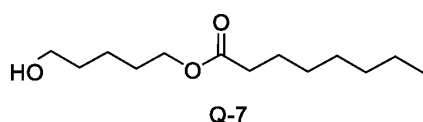
10 **Intermediate [Q-4]:**



- [0688]** To a stirred solution of tert-butyl (2-(dimethylamino)ethyl) succinate **[Q-3]** (9 g, 0.036 mol) in DCM (100 mL), Trifluoroacetic acid (10 mL, 0.132 mol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was
- 15 monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with triethylamine upto pH 7. The resulting organic layer concentrated under reduced pressure and the crude directly used in next step.

[0689] ELSD analysis: Purity 99.15 %, Calculated C₈H₁₅NO₄ = 189.10, Observed = 189.80 (m/z, M+H⁺).

20 **Intermediate [Q-7]:**



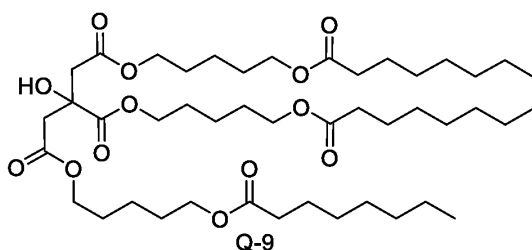
- [0690]** To a stirred solution of octanoic acid **[Q-6]** (10 g, 69.3 mmol) and pentane-1,5-diol **[Q-5]** (6.5 g, 62.4 mmol) in dichloromethane (200 mL, 781 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (16.7 g, 87.4 mmol) was
- 25 added followed by 4-(dimethylamino)pyridin-1-ium (1.71 g, 13.9 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture, and extracted with DCM (3x500 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the

crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl octanoate **[Q-7]** (6.0 g, 26.0 mmol, 37.56% yield) as yellow liquid.

[0691] ¹H-NMR (400 MHz, CDCl₃)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 4H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

5

Intermediate [Q-9]:

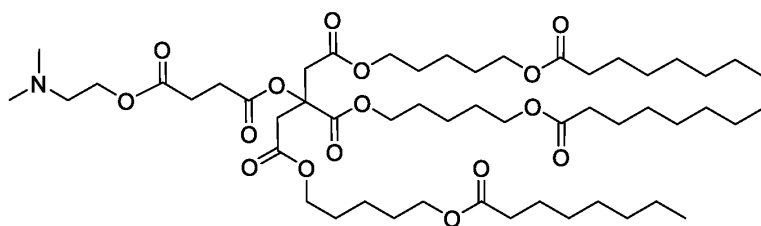


[0692] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid **[Q-8]** (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate **[Q-7]** (6 g, 26 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired tris(5-octanoyloxy)pentyl 2-hydroxypropane-1,2,3-tricarboxylate **[Q-9]** (2.1 g, 2.53 mmol, 38.9% yield) as yellow liquid.

[0693] ¹H-NMR (400 MHz, CDCl₃)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, *J* = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H).

[0694] *ELSD analysis:* Purity 97.90 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.35 (*m/z*, M+H⁺).

20

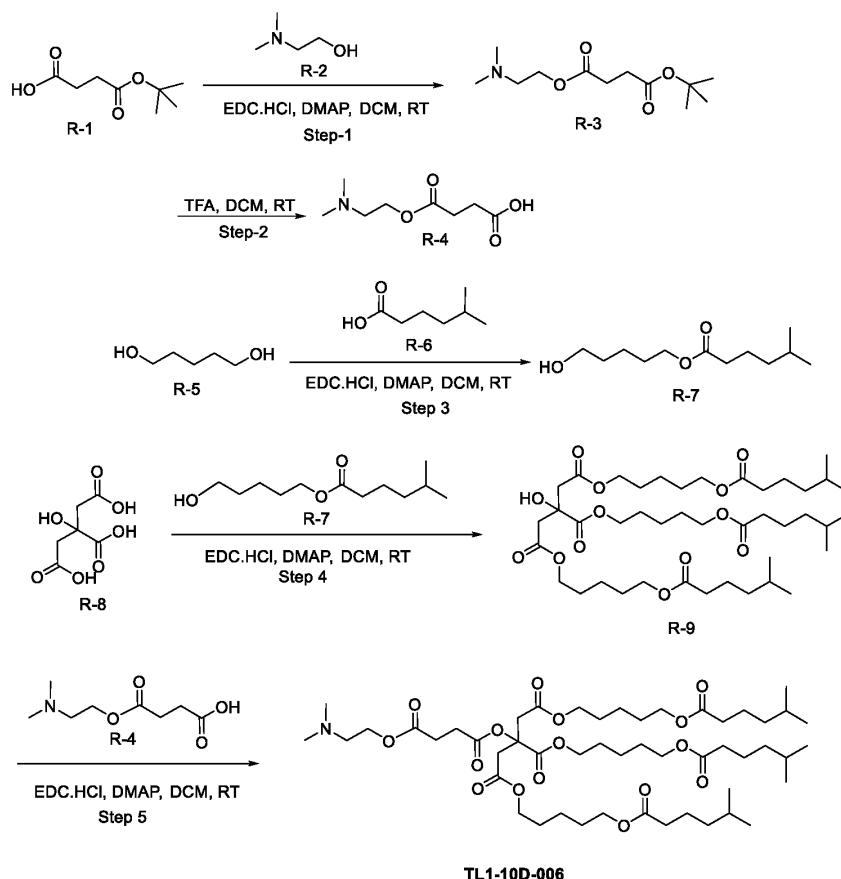
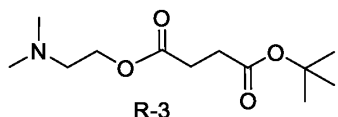
TL1-12D-001 (Compound (33))

(Compound (33))

- [0695]** To a stirred solution of 4-[2-(dimethylamino)ethoxy]-4-oxobutanoic acid [**Q-4**] (1.92 g, 10.1 mmol) and 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [**Q-9**] (2.1 g, 2.53 mmol) in dichloromethane (15 mL, 234 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (1.94 g, 10.1 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (312 mg, 2.53 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexane), to give the desired product [TL1-12D-001] (Compound (33)) (115 mg, 4.54 % yield) as a transparent liquid.

- [0696]** ¹H-NMR (400 MHz, DMSO-d₆)- δ 4.08-3.97 (m, 14H), 3.17-3.13 (d, *J* = 15.6 Hz, 2H), 3.10-3.06 (d, *J* = 15.2 Hz, 2H), 2.46-2.43 (m, 6H), 2.27-2.24 (m, 6H), 2.14 (s, 6H), 1.58-1.48 (m, 18H), 1.35-1.23 (m, 30H), 0.86-0.83 (m, 9H).

- [0697]** *ELSD analysis:* Purity 99.90 %, Calculated C₅₃H₉₃NO₁₆ = 999.65, Observed = 1000.50 (*m/z*, *M*+H⁺).

Example 18. Synthetic Protocol for M1266-497687 (TL1-10D-006) (Compound (27))**Synthetic Scheme R****Intermediate [R-3]:**

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10

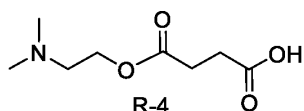
[0698] To a stirred solution of 4-(tert-butoxy)-4-oxobutanoic acid **[R-1]** (1.5 g, 0.0086 mol) and 2-(dimethylamino)ethan-1-ol **[R-2]** (0.92 g, 0.0103 mol) in dichloromethane (20 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (3.30 g, 0.0172 mol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.0 g, 0.0086 mol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (20 mL) was added to the reaction mixture, and extracted with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in

Dichloromethane), to give the desired tert-butyl (2-(dimethylamino)ethyl) succinate **[R-3]** (1.5 g, 71% yield) as pale yellow liquid.

[0699] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.16 (t, J = 6.0 Hz, 2H), 2.59-2.48 (m, 6H), 2.24 (s, 6H), 1.41 (s, 9H)

5

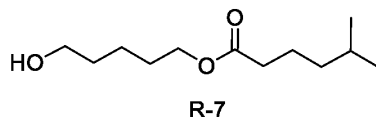
Intermediate [R-4]:



[0700] To a stirred solution of tert-butyl (2-(dimethylamino)ethyl) succinate **[R-3]** (1.5 g, 0.006 mol) in DCM (15 mL), Trifluoroacetic acid (1.4 g, 0.012 mol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with triethylamine upto pH 7. The resulting organic layer concentrated under reduced pressure and the crude directly used in next step.

[0701] *ELSD analysis:* Purity 99.15 %, Calculated $\text{C}_8\text{H}_{15}\text{NO}_4$ = 189.10, Observed = 189.80 (m/z , $\text{M}+\text{H}^+$).

Intermediate [R-7]:



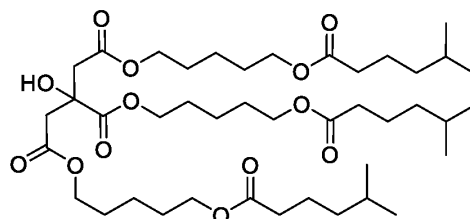
20

[0702] To a stirred solution of 5-methylhexanoic acid **[R-6]** (10 g, 76.8 mmol) and pentane-1,5-diol **[R-5]** (8 g, 76.8 mmol) in dichloromethane (150 mL) was cooled to 0 °C, EDC.HCl (18.6 g, 96.8 mmol), was added followed by 4-(dimethylamino)pyridin-1-ium (1.89 g, 15.4 mmol) and stirred for 16 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography using 0-20 % ethyl acetate in n-hexane to obtain pure product 5-hydroxypentyl 5-methylhexanoate **[R-7]** (6.1 g, 37%) as a pale yellow liquid.

25

[0703] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.07 (t, J = 6.4 Hz, 2H), 3.65 (t, J = 6.4 Hz, 2H), 2.27 (t, J = 7.2 Hz, 2H), 1.69-1.53 (m, 8H), 1.51-1.39 (m, 2H), 1.24-1.16 (m, 2H), 0.87 (d, J = 6.8 Hz, 6H).

Intermediate [R-9]:



R-9

5

[0704] To a stirred solution of 5-hydroxypentyl 5-methylhexanoate **[R-7]** (6 g, 27.7 mmol), and 2-hydroxypropane-1,2,3-tricarboxylic acid **[R-8]** (1.33 g, 6.93 mmol) in dichloromethane (150 mL) was cooled to 0 °C, EDC.HCl (5.32 g, 27.7 mmol), was added followed by 4-(dimethylamino)pyridin-1-ium (854 mg, 6.93 mmol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane), to obtain pure product 1,2,3-tris({5-[(5-methylhexanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[R-9]** (1.9 g, 35%) as a pale yellow liquid.

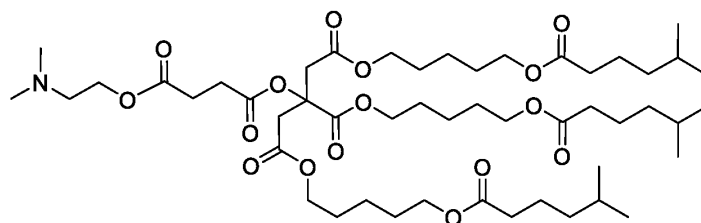
10

15

[0705] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.21 (t, J = 6.4 Hz, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.27 (t, J = 7.6 Hz, 6H), 1.68-1.51 (m, 22H), 1.41-1.38 (m, 6H), 1.21-1.16 (m, 6H), 0.88 (d, J = 6.4 Hz, 18H).

20

JC- TL1-10D-006 (Compound (27))



(Compound (27))

[0706] To a stirred solution of 1,2,3-tris({5-[(5-methylhexanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[R-9]** (1.9 g, 2.41 mmol) and 4-[2-(dimethylamino)ethoxy]-4-oxobutanoic acid

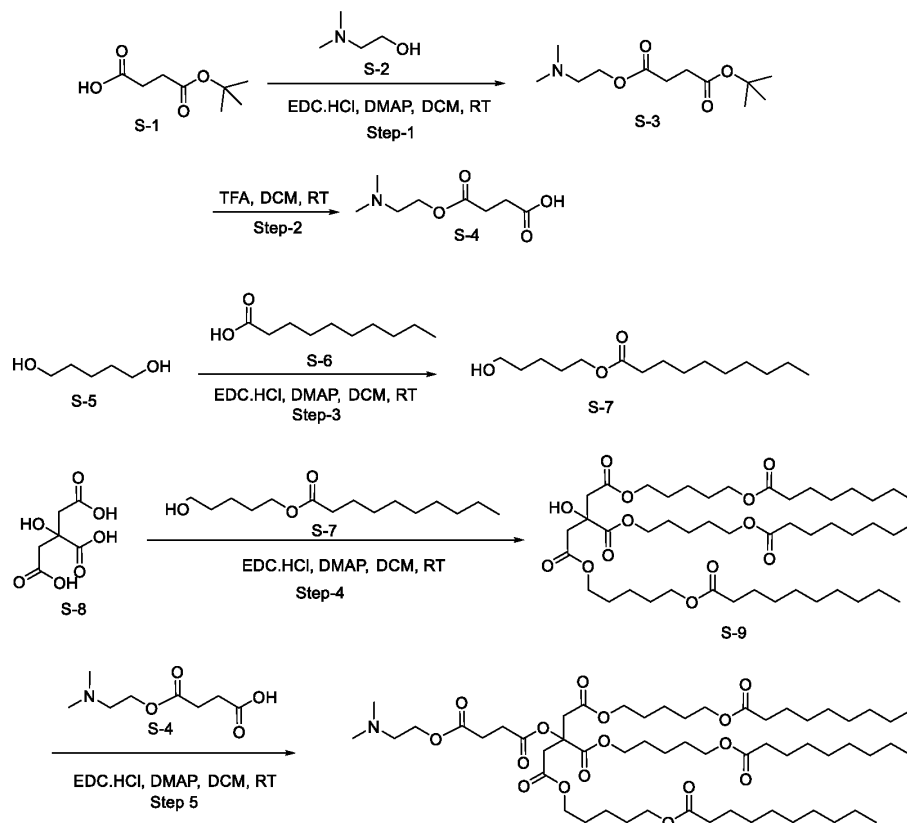
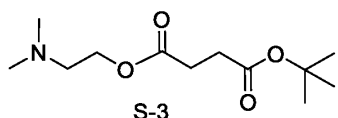
[R-4] (1.83 g, 9.66 mmol) in dichloromethane (20 mL) was cooled to 0 °C, EDC.HCl (1.85 g, 9.66 mmol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.247 g, 2.41 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (20 mL) was added to the reaction mixture and extracted with DCM (3x50 mL). The

5 resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product [JC- TL1-10D-006] (Compound (27)) (0.5 g, 22%) as a brown liquid.

[0707] ¹H-NMR (400 MHz, CDCl₃)- δ 4.19 (t, *J* = 4.8 Hz, 2H), 4.14 (t, *J* = 6.8 Hz, 2H), 4.09-4.04 (m, 10H), 3.30-3.19 (dd, *J* = 15.6, 12.8 Hz, 4H), 2.63-2.62 (m, 4H), 2.60-2.59 (br, 2H), 2.30-2.29 (m, 4H), 2.27-2.25 (m, 7H), 1.71-1.51 (m, 21H), 1.44-1.36 (m, 6H), 1.21-1.12 (m, 6H), 0.88 (d, *J* = 6.4 Hz, 18H).

10

ELSD analysis: Purity 99.89 %, Calculated C₅₀H₈₇NO₁₆ = 957.60, Observed = 958.45 (m/z, M+H⁺).

Example 19. Synthetic Protocol for M1266-499030 (TL1-14D-003) (Compound (24))**Synthetic Scheme S****Intermediate [S-3]:**

5

[0708] To a stirred solution of 4-(tert-butoxy)-4-oxobutanoic acid **[S-1]** (10 g, 0.0574 mol) and 2-(dimethylamino)ethan-1-ol **[S-2]** (6.14 g, 0.0689 mmol) in dichloromethane (100 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (13.9 g, 0.0723 mol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.41 g, 0.0115 mol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in

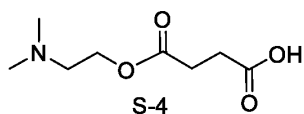
10

Dichloromethane), to give the desired tert-butyl (2-(dimethylamino)ethyl) succinate [**S-3**] (9.0 g, 63.91% yield) as pale yellow liquid.

Results:

- 5 **[0709]** $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.16 (t, J = 6.0 Hz, 2H), 2.59-2.41 (m, 6H), 2.24 (s, 6H), 1.41 (s, 9H)

Intermediate [S-4]:

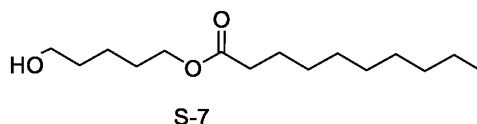


- 10 **[0710]** To a stirred solution of tert-butyl (2-(dimethylamino)ethyl) succinate [**S-3**] (9 g, 0.036 mol) in DCM (100 mL), Trifluoroacetic acid (10 mL, 0.132 mol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with triethylamine upto pH 7. The resulting organic layer concentrated under reduced pressure and the
- 15 crude directly used in next step.

Results:

- 20 *ELSD analysis:* Purity 99.15 %, Calculated $\text{C}_8\text{H}_{15}\text{NO}_4$ = 189.10, Observed = 189.80 (m/z , $\text{M}+\text{H}^+$).

Intermediate [S-7]:

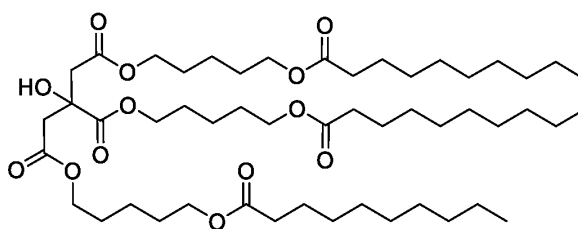


- [0711]** To a stirred solution of decanoic acid [**S-6**] (10 g, 58 mmol) and pentane-1,5-diol [**5**] (5.44 g, 52.2 mmol) in dichloromethane (200 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (14 g, 73.1 mmol) was
- 25 added followed by 4-(dimethylamino)pyridin-1-ium (1.43 g, 11.6 mmol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture, and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure, and the crude
- 30 was purified by flash column chromatography (SiO_2 : 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl decanoate [**S-7**] (3.6 g, 24% yield) as yellow liquid.

Results:

¹H-NMR (400 MHz, CDCl₃)- δ 4.09 (t, *J* = 6.4 Hz, 2H), 3.67 (q, *J* = 6.0 Hz, 2H), 2.30 (t, *J* = 7.6 Hz, 2H),
 5 1.70-1.56 (m, 6H), 1.49-1.43 (m, 2H), 1.31-1.23 (m, 12H), 0.91 (m, 3H).

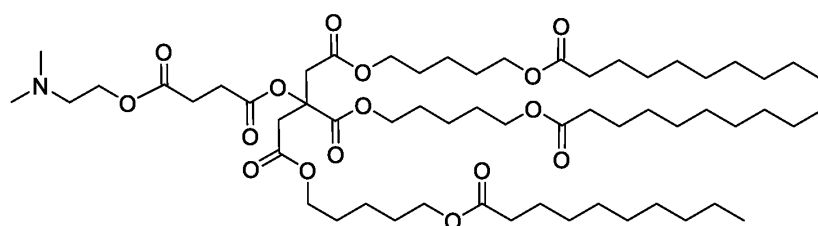
ELSD analysis: Purity 99.43 %, Calculated C₁₅H₃₀O₃ = 258.22, Observed = 259.15 (m/z, M+H⁺).

Intermediate [S-9]:**S-9**

10 **[0712]** To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid **[S-8]** (651 mg, 3.39 mmol) and 5-hydroxypentyl decanoate **[S-7]** (3.5 g, 13.5 mmol) in dichloromethane (50 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (2.6 g, 13.5 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (417 mg, 3.39 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by TLC (SM was
 15 consumed). Water (20 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 1,2,3-tris[5-(decanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate **[S-9]** (1.97 g, 2.16 mmol) as pale yellow liquid.

20 **[0713]** ¹H-NMR (400 MHz, CDCl₃)- δ 4.22 (t, *J* = 6.4 Hz, 2H), 4.11-4.04 (m, 10H), 2.88 (dd, *J* = 15.6, 19.2 Hz, 4H), 2.28 (t, *J* = 7.6 Hz, 6H), 1.73-1.59 (m, 18H), 1.43-1.36 (m, 6H), 1.29-1.26 (m, 36H), 0.88 (d, *J* = 6.8 Hz, 9H).

[0714] *ELSD analysis:* Purity 98.31%, Calculated C₅₁H₉₂O₁₃ = 912.65, Observed = 935.90 (m/z, M+Na⁺).

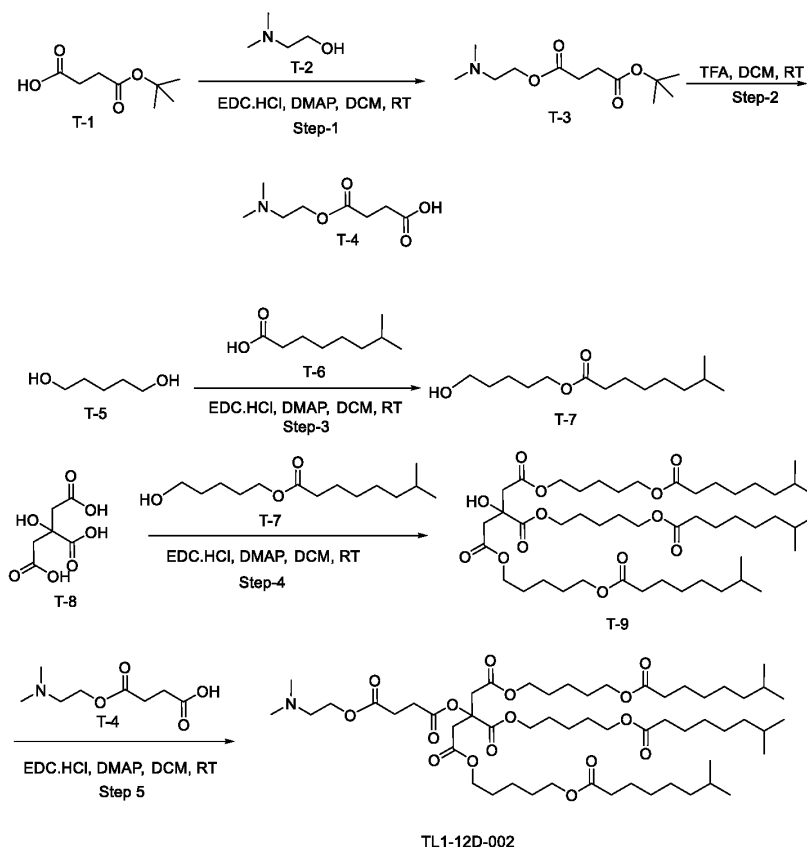
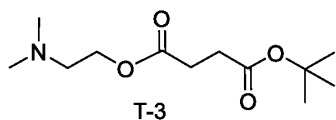
JC-TL1-14D-003 (Compound (24))

(Compound (24))

- [0715] To a stirred solution of 4-[2-(dimethylamino)ethoxy]-4-oxobutanoic acid [**S-4**] (1.51 g, 8.32 mmol) and 1,2,3-tris[5-(decanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [**S-9**] (1.9 g, 2.41 mmol), in dichloromethane (50 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (1.6 g, 8.32 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (254 mg, 2.08 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed).
- 10 Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexane), to give the desired product [JC-TL1-14D-003] (Compound (24)) (0.6 g, 26%) as a yellow oil

- [0716] ¹H-NMR (400 MHz, CDCl₃)- δ 4.20 (t, *J* = 5.6 Hz, 2H), 4.14 (t, *J* = 6.8 Hz, 2H), 4.09-4.03 (m, 10H), 3.28 (d, *J* = 15.6 Hz, 2H), 3.21 (d, *J* = 15.6 Hz, 2H), 2.65-2.60 (m, 6H), 2.30-2.26 (m, 12H), 1.71-1.56 (m, 18H), 1.44-1.36 (m, 6H), 1.29-1.25 (m, 36H), 0.87 (d, *J* = 6.8 Hz, 9H).
- 15

[0717] *ELSD analysis*: Purity 99.72 %, Calculated C₅₉H₁₀₅NO₁₆ = 1083.74, Observed = 1084.50 (m/z, M+H⁺).

Example 20. Synthetic Protocol for M1266-507720 (TL1-12D-002) (Compound (35))**Synthetic Scheme T****5 Intermediate [T-3]:**

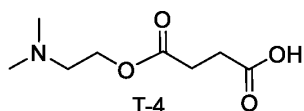
- [0718]** To a stirred solution of 4-(tert-butoxy)-4-oxobutanoic acid [**T-1**] (10 g, 0.0574 mol) and 2-(dimethylamino)ethan-1-ol [**T-2**] (6.14 g, 0.0689 mmol) in dichloromethane (100 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (13.9 g, 0.0723 mol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.41 g, 0.0115 mol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in

Dichloromethane), to give the desired tert-butyl (2-(dimethylamino)ethyl) succinate **[T-3]** (9.0 g, 63.91% yield) as yellow liquid.

[0719] ¹H-NMR (400 MHz, CDCl₃)- δ 4.16 (t, *J* = 6.0 Hz, 2H), 2.59-2.41 (m, 6H), 2.24 (s, 6H), 1.41 (s, 9H)

5

Intermediate [T-4]:

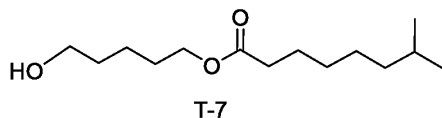


[0720] To a stirred solution of tert-butyl (2-(dimethylamino)ethyl) succinate **[T-3]** (9 g, 0.036 mol) in DCM (100 mL), Trifluoroacetic acid (10 mL, 0.132 mol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with triethylamine upto pH 7. The resulting organic layer concentrated under reduced pressure and the crude directly used in next step.

[0721] *ELSD analysis:* Purity 99.15 %, Calculated C₈H₁₅NO₄ = 189.10, Observed = 189.80 (*m/z*, M+H⁺).

15

Intermediate [T-7]:



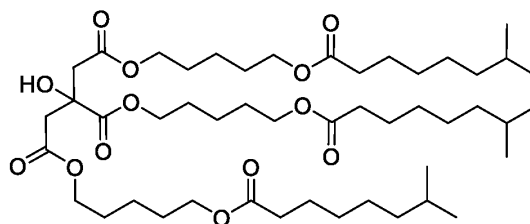
[0722] To a stirred solution of 7-methyloctanoic acid **[T-6]** (5 g, 31.6 mmol) and pentane-1,5-diol **[T-5]** (3.29 g, 31.6 mmol) in dichloromethane (100 mL, 781 mmol) was cooled to 0 °C, *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (7.63 g, 39.8 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (778 mg, 6.32 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x200 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-20% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl 7-methyloctanoate **[T-7]** (4.4 g, 57% yield) as yellow liquid.

25

[0723] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.06 (t, J = 6.4 Hz, 2H), 3.65 (t, J = 6.4 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.67-1.57 (m, 6H), 1.52-1.40 (m, 4H), 1.30-1.26 (m, 4H), 1.15-1.14 (m, 2H), 0.85 (d, J = 6.8, 6H).

5

Intermediate [9]:



T-9

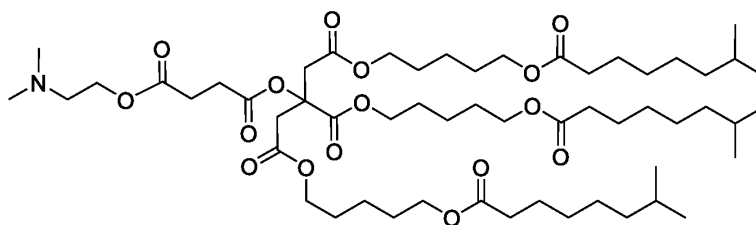
[0724] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid **[T-8]** (0.6 g, 3.12 mmol) and 5-hydroxypentyl 7-methyloctanoate **[T-7]** (3.05 g, 12.5 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, EDC hydrochloride (2.39 g, 12.5 mmol), was added followed by 4-(dimethylamino)pyridin-1-ium (385 mg, 3.12 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired 1,2,3-tris({5-[(7-methyloctanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[T-9]** (1 g, 36.75% yield) as pale yellow liquid.

15

[0725] *ELSD analysis*: Purity 99.50 %, Calculated $\text{C}_{48}\text{H}_{86}\text{O}_{13}$ = 870.61, Observed = 893.35 (m/z , $\text{M}+\text{Na}^+$).

20

TL1-12D-002 (Compound (35))

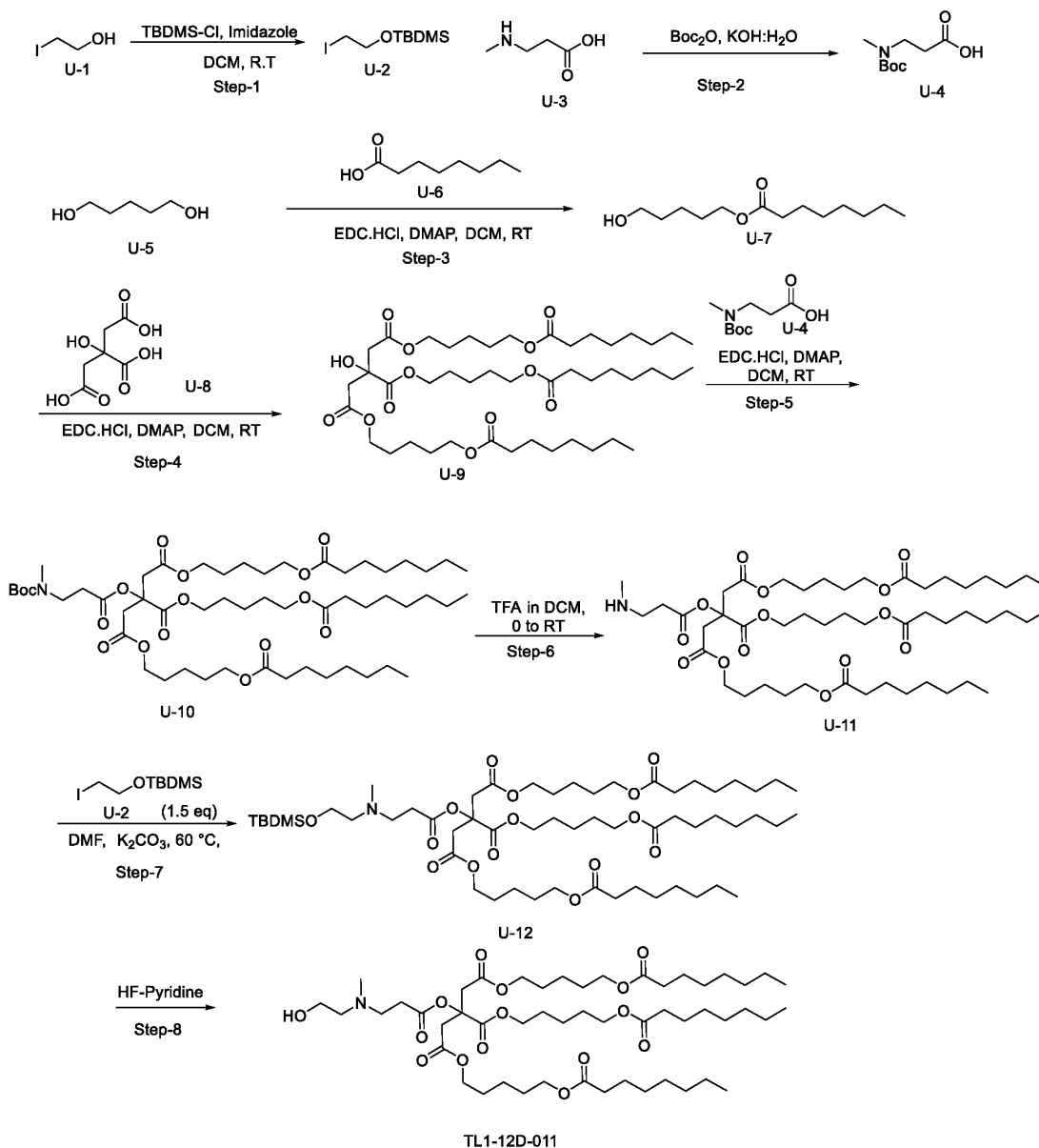
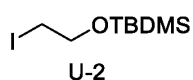


Compound (35)

[0726] To a stirred solution of 4-[2-(dimethylamino)ethoxy]-4-oxobutanoic acid **[T-4]** (869 mg, 4.59 mmol) and 1,2,3-tris({5-[(7-methyloctanoyl)oxy]pentyl}) 2-hydroxypropane-1,2,3-tricarboxylate **[T-9]** (1 g, 1.15 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, EDC hydrochloride (880 mg, 4.59 mmol), was added followed by 4-(dimethylamino)pyridin-1-ium
5 (141 mg, 1.15 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired product [TL1-12D-
10 002] (Compound (35))(140 mg, 11.7% yield) as pale yellow liquid.

[0727] ¹H-NMR (400 MHz, CDCl₃)- δ 4.20 (t, *J* = 5.6 Hz, 2H), 4.15-4.12 (m, 2H), 4.09-4.04 (m, 10H), 3.28 (d, *J* = 15.6 Hz, 2H), 3.21 (d, *J* = 15.6 Hz, 2H), 2.65-2.62 (m, 6H), 2.31-2.26 (m, 10H), 1.71-1.56 (m, 18H), 1.52-1.46 (m, 3H), 1.44-1.36 (m, 6H), 1.32-1.25 (m, 14H), 1.18-1.10 (m, 6H), 0.85 (d, *J* = 6.4, 18H).

15 **[0728]** *ELSD analysis*: Purity 99.89 %, Calculated C₅₆H₉₉NO₁₆ = 1041.70, Observed = 1042.50 (m/z, M+H⁺).

Example 21. Synthetic Protocol for M1266-534954 (TL1-12D-011) (Compound 55)**Synthetic Scheme U****Intermediate [U-2]:**

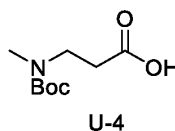
5

[0729] To a stirred solution of 2-iodoethan-1-ol [**U-1**] (5 g, 0.0290 mol) in dichloromethane (100 mL), 1*H*-imidazole (1.97 g, 0.0290 mol) was added at room temperature. The resulting

reaction mixture was cooled to 0 °C and tert-butyl(chloro)dimethylsilane (8.76 g, 0.0580 mol) was added portion wise at same temperature, then allowed to stirred for 16 h at room temperature. Progress of reaction was monitored by ELSD/TLC. Water (50 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-10 % ethyl acetate in n-hexane), to obtain pure product **[2]** (5.0 g, 60.1 %, Yield) as a colourless liquid.

[0730] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 3.83 (t, J = 5.6 Hz, 2H), 3.19 (t, J = 6.8 Hz, 2H), 0.90 (s, 9H), 0.10 (s, 6H).

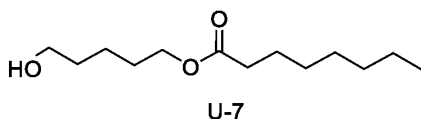
Intermediate [3]:



[0731] To a stirred solution of 3-(methylamino)propanoic acid **[U-3]** (5 g, 48.5 mmol), and potassium hydroxide (5.44 g, 97 mmol) in 1,4-dioxane (25 mL, 293 mmol) and water (25 mL, 1.39 mol) added di-tert-butyl dicarbonate (10.6 g, 48.5 mmol) at room temperature for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, mixture was concentrated under reduced pressure. The crude material was acidified by 10 % NaHSO_4 upto pH 2-3, and extracted with ethyl acetate (3x200 mL). The resulting organic layer was dried over anhydrous sodium sulphate concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product 3-[[[(tert-butoxy)carbonyl](methyl)amino]propanoic acid **[U-4]** (9 g, 91.33 %, Yield) as a colourless liquid.

[0732] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 3.50 (t, J = 6.8 Hz, 2H), 2.87 (s, 3H), 2.59 (bs, 2H), 1.48 (s, 9H).

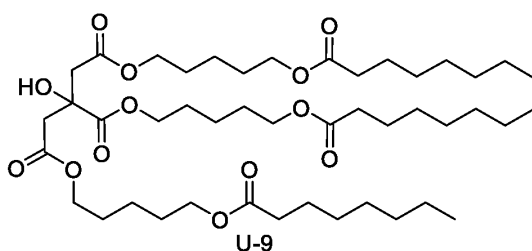
Intermediate [U-7]:



[0733] To a stirred solution of octanoic acid [U-6] (10 g, 69.3 mmol) and pentane-1,5-diol [U-5] (6.5 g, 62.4 mmol) in dichloromethane (200 mL, 781 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (16.7 g, 87.4 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.71 g, 13.9 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (100 mL) was added to the reaction mixture, and extracted with DCM (3x300 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl octanoate [U-7] (6.0 g, 26.0 mmol, 37.56% yield) as pale yellow liquid.

[0734] ¹H-NMR (400 MHz, CDCl₃)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 4H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

Intermediate [U-9]:



15

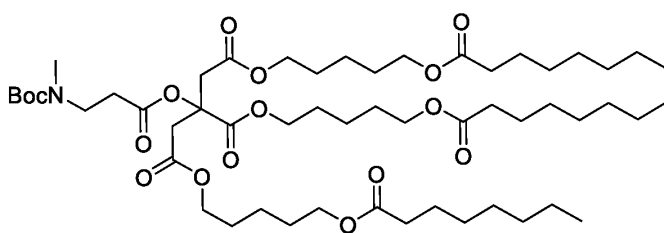
[0735] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [U-8] (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate [U-7] (6 g, 26 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired tris(5-octanoyloxy)pentyl 2-hydroxypropane-1,2,3-tricarboxylate [U-9] (2.1 g, 38.9% yield) as pale yellow liquid.

25

[0736] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.89-0.85 (m, 9H).

[0737] *ELSD analysis*: Purity 97.90 %, Calculated $\text{C}_{45}\text{H}_{80}\text{O}_{13}$ = 828.56, Observed = 829.35 (m/z , $\text{M}+\text{H}^+$).

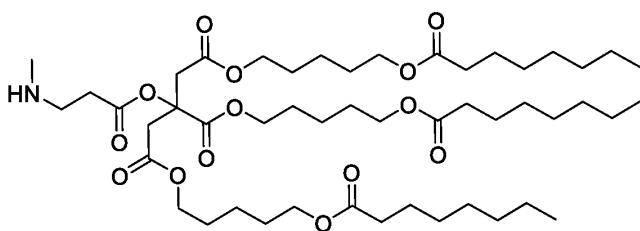
Intermediate [U-10]:



U-10

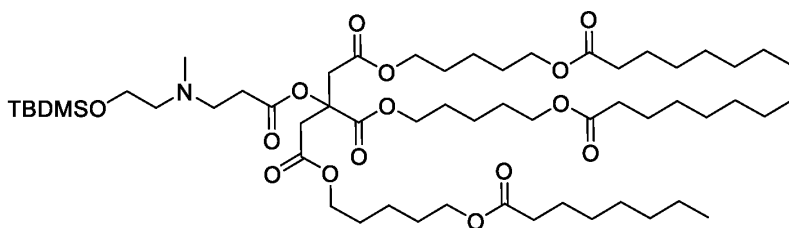
[0738] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [9] (2.96 g, 3.57 mmol) and 3-[[[(tert-butoxy)carbonyl](methyl)amino]propanoic acid [U-4] (2.9 g, 14.3 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (2.74 g, 14.3 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (439 mg, 3.57 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[[(tert-butoxy)carbonyl](methyl)amino]propanoyl]oxy]propane-1,2,3-tricarboxylate [U-10] (3 g, 82.5 % yield) as a light yellow liquid.

[0739] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.21-4.04 (m, 12H), 3.47 (t, J = 7.2Hz, 2H), 3.28 (d, J = 15.6Hz, 2H), 3.20 (d, J = 15.6Hz, 2H), 2.83 (bs, 3H), 2.56 (t, J = 7.2Hz, 2H), 2.28 (t, J = 7.2Hz, 6H), 1.71-1.59 (m, 18H), 1.47-1.39 (m, 15H), 1.36-1.27 (m, 24H), 0.89-0.85 (m, 9H).

Intermediate [U-11]:**U-11**

[0740] Trifluoroacetic acid (4.98 mL, 65.1 mmol) was added into a solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[[tert-butoxy]carbonyl](methyl)amino]propanoyl]oxy]propane-1,2,3-tricarboxylate **[U-10]** (3 g, 2.96 mmol) in dichloromethane (40 mL, 625 mmol) at 0 °C, allow to stirred for until reaction completion. The progress of reaction was monitored by TLC. After completion the reaction, reaction mixture was concentrated under reduced pressure to remove excess trifluoroacetic acid. The reaction mass was quenched with saturated NaHCO₃ solution up to pH 8, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in Dichloromethane), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[U-11]** (2.85 g, 93 % yield) as pale yellow liquid.

[0741] ¹H-NMR (400 MHz, CDCl₃) - δ 4.21-4.16 (m, 2H), 4.10-4.04 (m, 10H), 3.25-3.16 (m, 6H), 2.85-2.81 (m, 3H), 2.72 (s, 3H), 2.28 (t, *J* = 7.6 Hz, 6H), 1.70-1.59 (m, 18H), 1.43 (m, 6H), 1.29-1.27 (m, 24H), 0.87 (t, *J* = 6.8 Hz, 9H).

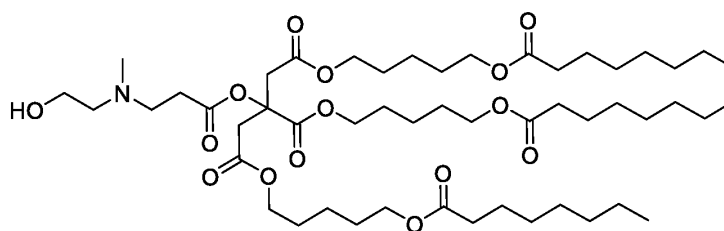
Intermediate [U-12]:**U-12**

[0742] To stirred a solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[U-11]** (1 g, 1.09 mmol) in acetonitrile (15 mL, 287 mmol), dipotassium carbonate (756 mg, 5.47 mmol), and tert-butyl(2-

iodoethoxy)dimethylsilane **[U-2]** (470 mg, 1.64 mmol) was added at room temperature, allow to stir at 60 °C for 16 h. The progress of reaction was monitored by TLC and ELSD data. After completion the reaction, reaction mass was cooled, filter by sintered glass funnel. The filtrate was concentrated under reduced pressure and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in Dichloromethane), to give the desired compound **[U-12]** (0.4 g, 34% yield) as pale yellow liquid.

[0743] *ELSD analysis:* Purity 99.58 %, Calculated C₅₇H₁₀₅NO₁₅Si = 1071.73, Observed = 1072.90 (m/z, M+H⁺).

TL1-12D-011



10

[0744] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[[2-[(tert-butyl)dimethylsilyl]oxy]ethyl](methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[U-12]** (0.4 g, 373 μmol) in tetrahydrofuran (10 mL, 123 mmol), hydrogen pyridine fluoride (202 μL, 2.24 mmol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with cold aqueous sodium bicarbonate solution upto pH 8, and extract with ethyl acetate (3x50 mL). The resulting organic layer was dried over Na₂SO₄, concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10 % methanol in dichloromethane), to obtain the desired produced **TL1-12D-011** (0.2 g, 56% yield) as a light yellow colour liquid.

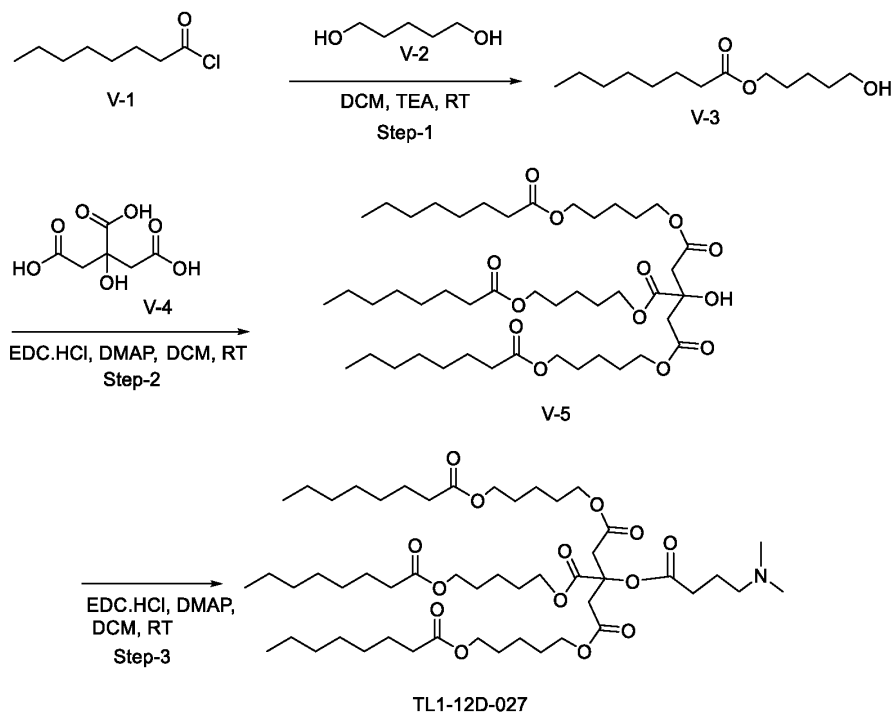
20

Results:

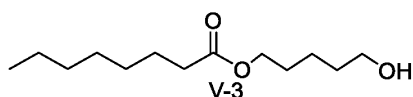
[0745] ¹H-NMR (400 MHz, CDCl₃)- δ 4.16 (t, J = 6.4 Hz, 2H), 4.09-4.04 (m, 10H), 3.60 (t, J = 5.2Hz, 2H), 3.29 (d, J = 15.6 Hz, 2H), 3.20 (d, J = 15.6Hz, 2H), 2.75 (t, J = 6.8Hz, 2H), 2.57-2.50 (m, 4H), 2.30-2.26 (m, 8H), 1.72-1.59 (m, 20H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.87 (t, J = 6.8Hz, 9H).

25

[0746] *ELSD analysis:* Purity 99.70 %, Calculated C₅₁H₉₁NO₁₅ = 957.64, Observed = 958.25 (m/z, M+H⁺).

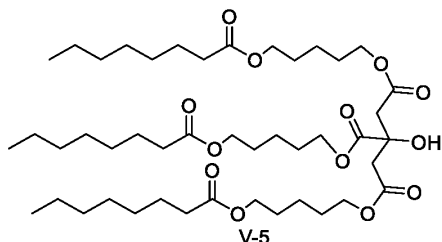
Example 22. Synthetic Protocol for M1266-539235 (TL1-12D-027) (Compound 75)**Synthetic Scheme V**

5

Compound V-3

[0747] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N, N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxyoctanoate [V-3] (16 g, 69.5 mmol) as light yellow colour liquid.

[0748] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

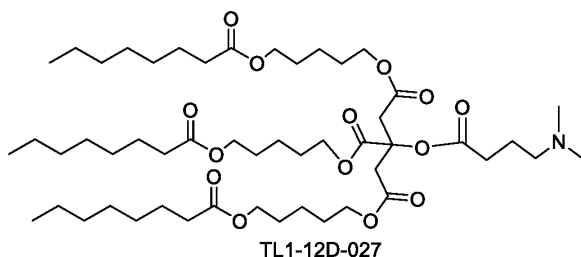
Compound V-5

[0749] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [V-3] (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass

10 diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [V-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

[0750] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, $J = 6.4$ Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, $J = 15.6$ Hz, 4H), 2.28 (t, $J = 7.6$ Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (7, $J = 7.2$ Hz, 9H).

15

TL1-12D-027 (Compound (75))

Compound (75)

20

[0751] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate (1 g, 1.21 mmol) and 4-(dimethylamino)butanoic acid (633 mg, 4 eq., 4.82 mmol) in dichloromethane (25.0 mL) was added EDCI (925 mg, 4 eq., 4.82 mmol) and N,N-dimethylpyridin-

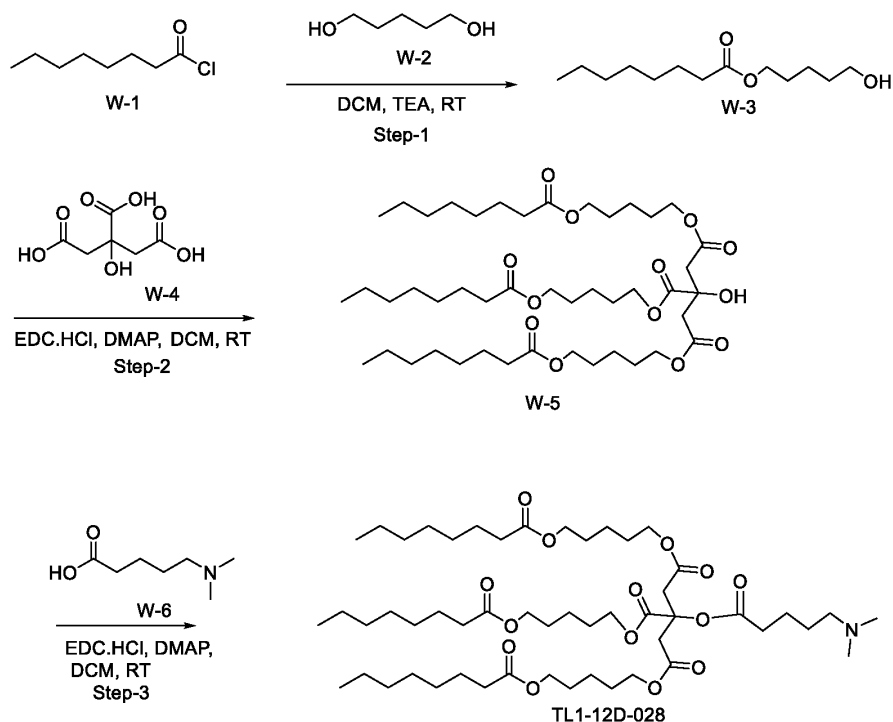
4-amine (147 mg, 1.21 mmol) at 0°C. Reaction mass allowed to RT and stirred for 16 hr. Progress of reaction was monitored by ELSD/TLC. To the resulting reaction mixture water (100 ml) was added and extracted with DCM (2x50.0 mL), dried over anhydrous sodium sulphate and concentrated. Crude was purified over silica using 3% MeOH in DCM to obtain 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[4-(dimethylamino)butanoyl]oxypropane-1,2,3-tricarboxylate [TL1-12D-027] (Compound (75)) (68 µg, 7.22e-8 mol) as colourless liquid mass.

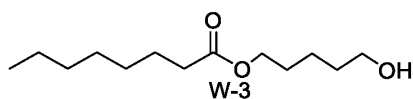
[0752] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, $J = 6.4$ Hz, 2H), 4.07 (m, 10H), 3.29-3.25 (d, $J = 15.6$ Hz, 2H), 3.21-3.17 (d, $J = 15.6$ Hz, 2H), 2.50 (m, 16H), 1.83 (bs, 2H), 1.61 (m, 14H), 1.40 (m, 6H), 1.27 (brs, 28H), 0.87 (t, $J = 10.8$ Hz, 9H).

[0753] LCMS analysis: Purity 99.35 %, Calculated $\text{C}_{51}\text{H}_{91}\text{NO}_{14} = 941.64$, Observed = 942.35 (m/z , $\text{M}+\text{H}^+$).

15 **Example 23. Synthetic Protocol for M1266-536156 (TL1-12D-028) (Compound (50))**

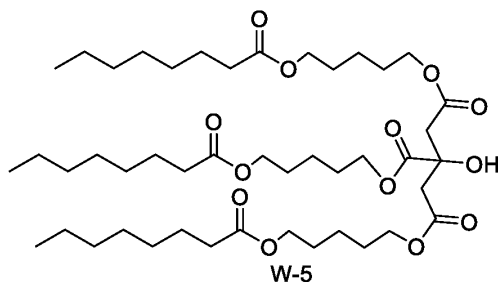
Synthetic Scheme W



Compound W-3

[0754] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N,N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxypentyl octanoate [W-3] (16 g, 69.5 mmol) as light yellow colour liquid.

[0755] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 6H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

Compound W-5

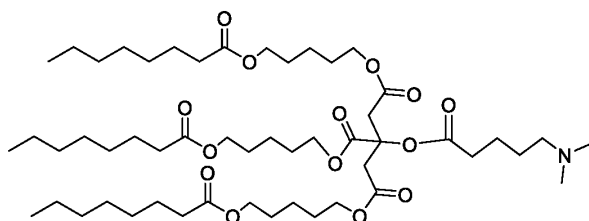
15

[0756] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [W-3] (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [W-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

25

[0757] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (7, J = 7.2 Hz, 9H).

TL1-12D-028 (Compound (50))



TL1-12D-028

Compound (50)

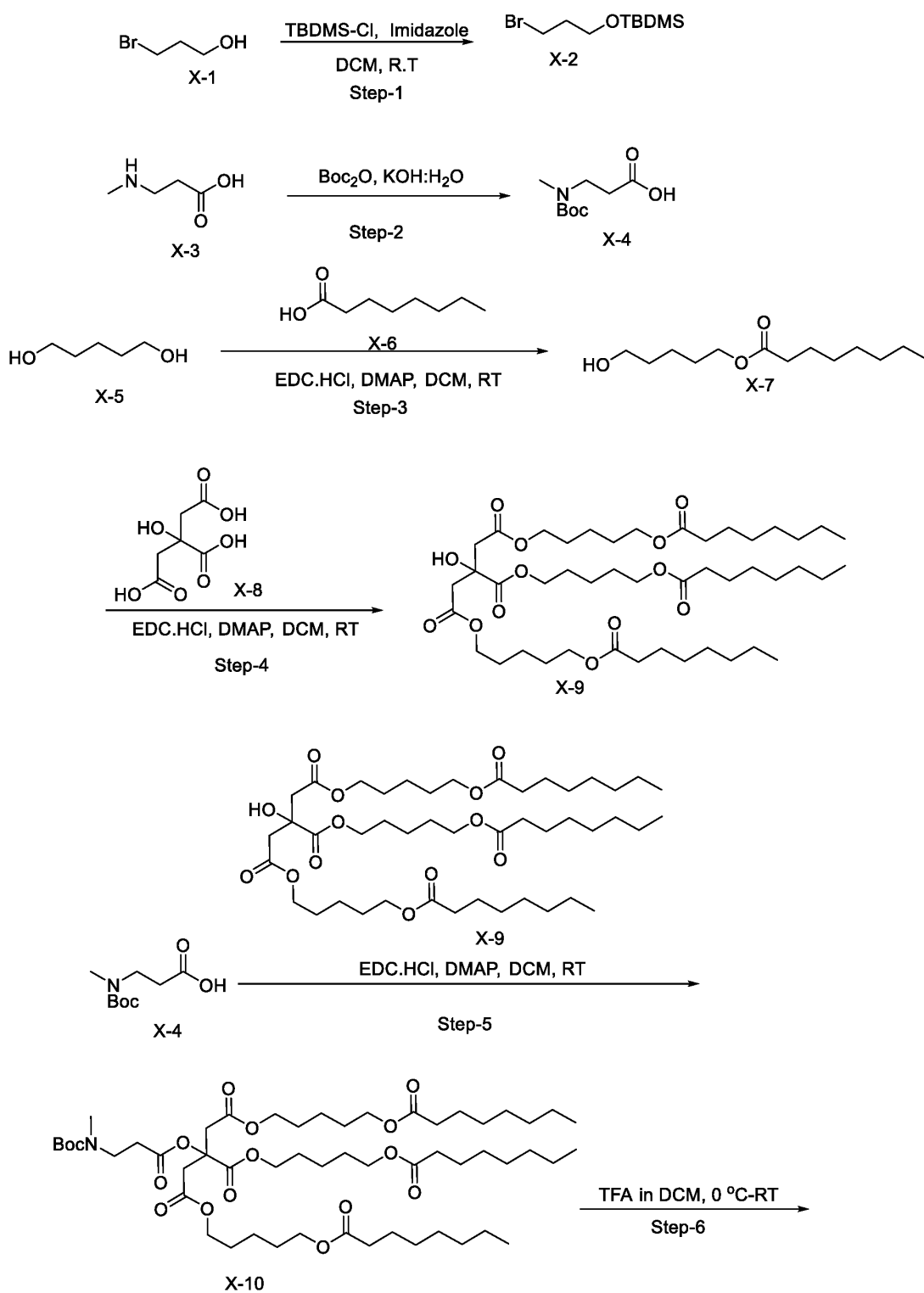
[0758] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [Int-5] (0.2 g, 241 μmol) in dichloromethane (10 mL) was added 5-(dimethylamino)pentanoic acid (35 mg, 241 μmol), EDCI (185 mg, 4 eq., 965 μmol) and N,N-dimethylpyridin-4-amine (29.5 mg, 241 μmol) at RT. Reaction mass was stirred for 16 h at RT. Progress of reaction mass was monitored by ELSD and TLC. Reaction mass was diluted with 25.0 mL of DCM and washed with water (25.0 mL). Organic layer was dried over anhydrous sodium sulphate and concentrated. Crude was purified over silica using 3% MeOH in DCM to obtain pure product [TL1-12D-028] (Compound (50)) (120 mg, 125 μmol) as brown colour mass.

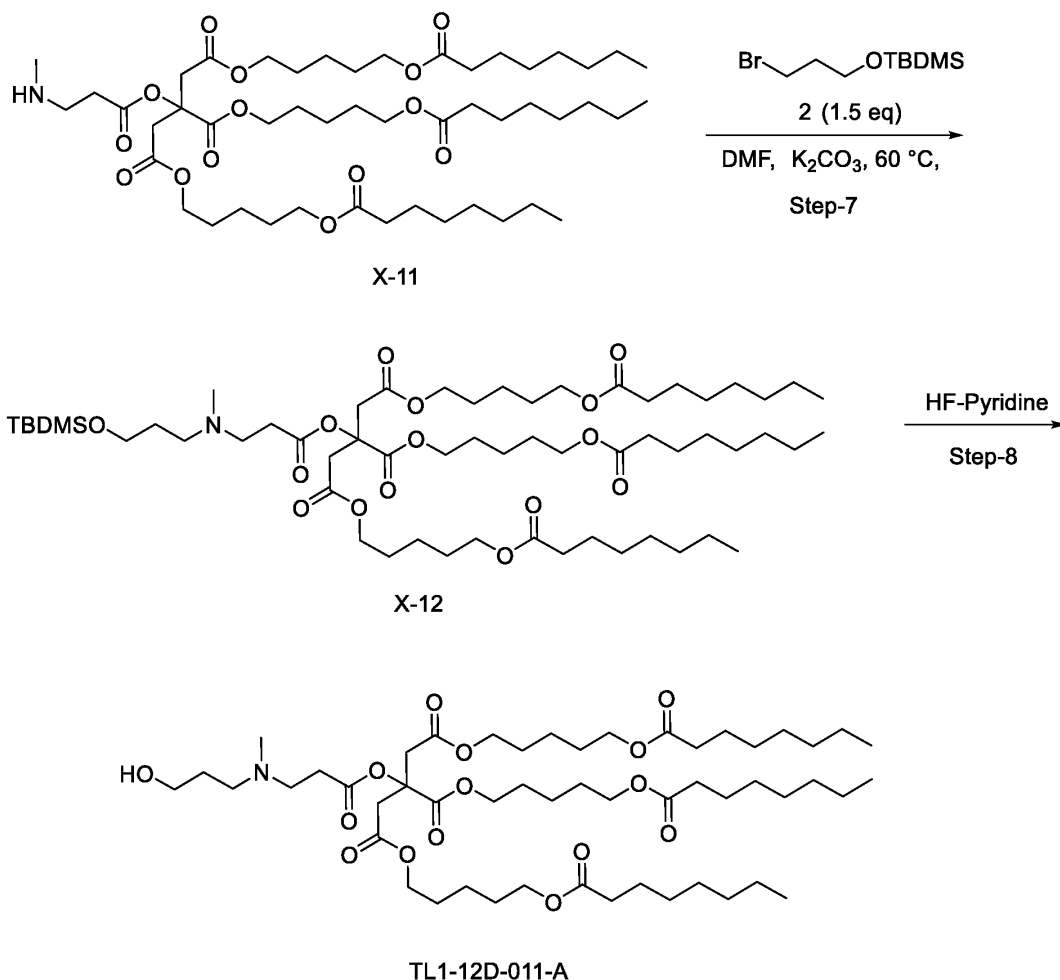
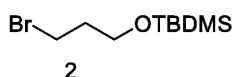
[0759] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, J = 6.8 Hz, 2H), 4.074 (m, 10H), 3.29-3.25 (d, J = 15.6 Hz, 2H), 3.21-3.17 (d, J = 15.6 Hz, 2H), 2.44 (bs, 8H), 2.30 (t, J = 7.2 Hz, 6H), 1.65 (m, 18H), 1.40 (m, 6H), 1.27 (brs, 28H), 0.87 (t, J = 2.8 Hz, 9H).

[0760] LCMS analysis: Purity 99.83 %, Calculated $\text{C}_{52}\text{H}_{93}\text{NO}_{14}$ = 955.66, Observed = 956.35 (m/z, $\text{M}+\text{H}^+$).

Example 24. Synthetic Protocol for M1266-548305 (TL1-12D-011-A) (Compound (62))

Synthetic Scheme X

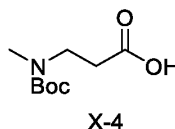


**Intermediate [X-2]:**

- [0761]** To a stirred solution of 3-bromopropan-1-ol [X-1] (5 g, 36 mmol) in dichloromethane (100 mL), 1*H*-imidazole (4.9 g, 71.9 mmol) was added at room temperature. The resulting reaction mixture was cooled to 0 °C and tert-butyl(chloro)dimethylsilane (10.8 g, 71.9 mmol) was added portion wise at same temperature, then allowed to stirred for 16 h at room temperature. Progress of reaction was monitored by ELSD/TLC. Water (50 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-10 % ethyl acetate in n-hexane), to obtain pure product [X-2] (4.1 g, 45 %, Yield) as a colourless liquid.

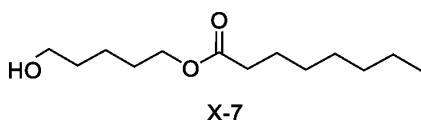
[0762] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 3.73 (t, J = 6.0 Hz, 2H), 3.51 (t, J = 6.4 Hz, 2H), 2.06-2.00 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H).

Intermediate [X-4]:



- 5 [0763] To stirred a solution of 3-(methylamino)propanoic acid [X-3] (5 g, 48.5 mmol), and potassium hydroxide (5.44 g, 97 mmol) in 1,4-dioxane (25 mL, 293 mmol) and water (25 mL, 1.39 mol) added di-tert-butyl dicarbonate (10.6 g, 48.5 mmol) at room temperature for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, mixture was concentrated under reduced pressure. The crude material was acidified by 10 % NaHSO_4 upto pH
- 10 2-3. Water (20 mL) was added to the reaction mixture and extracted with ethyl acetate (3x100 mL). The resulting organic layer was dried over anhydrous sodium sulphate concentrated under reduced pressure to obtain crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain pure product 3-[[tert-butoxy)carbonyl](methyl)amino]propanoic acid [X-4] (9 g, 91.33 %, Yield) as a colourless liquid.
- 15 [0764] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 3.50 (t, J = 6.8 Hz, 2H), 2.87 (s, 3H), 2.59 (bs, 2H), 1.48 (s, 9H).

Intermediate [X-7]:



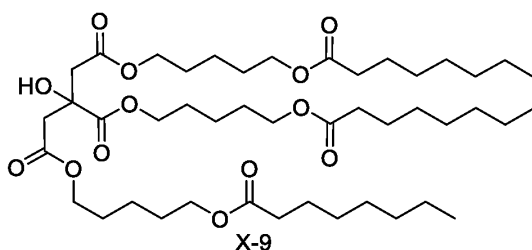
- 20 [0765] To a stirred solution of octanoic acid [X-6] (10 g, 69.3 mmol) and pentane-1,5-diol [X-5] (6.5 g, 62.4 mmol) in dichloromethane (200 mL, 781 mmol) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (16.7 g, 87.4 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.71 g, 13.9 mmol) and the reaction mixture stirred at r.t. for 16 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed).
- 25 Water (50 mL) was added to the reaction mixture, and extracted with DCM (2x200 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-10% Ethyl acetate in Hexane), to give

the desired 5-hydroxypentyl octanoate **[X-7]** (6.0 g, 26.0 mmol, 37.56% yield) as pale yellow liquid.

[0766] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 4H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

5

Intermediate [X-9]:

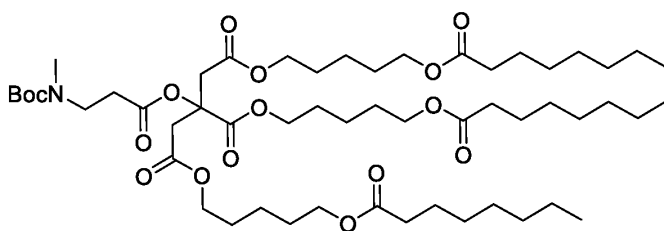


[0767] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid **[X-8]** (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate **[X-7]** (6 g, 26 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired tris(5-octanoyloxy)pentyl 2-hydroxypropane-1,2,3-tricarboxylate **[X-9]** (2.1 g, 38.9% yield) as pale yellow liquid.

[0768] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H).

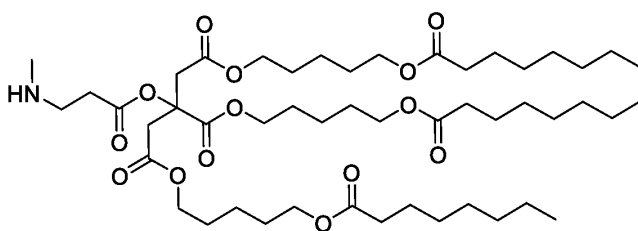
[0769] *ELSD analysis:* Purity 97.90 %, Calculated $\text{C}_{45}\text{H}_{80}\text{O}_{13}$ = 828.56, Observed = 829.35 (m/z , $\text{M}+\text{H}^+$).

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Intermediate [X-10]:**X-10**

[0770] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate **[X-9]** (2.96 g, 3.57 mmol) and 3-[[[(tert-butoxy)carbonyl](methyl)amino]propanoic acid **[X-4]** (2.9 g, 14.3 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C, {[3-(dimethylamino)propyl]imino}methylidene(ethyl)amine hydrochloride (2.74 g, 14.3 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (439 mg, 3.57 mmol) and the reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[[(tert-butoxy)carbonyl](methyl)amino]propanoyl]oxy]propane-1,2,3-tricarboxylate **[X-10]** (3 g, 82.5 % yield) as a light yellow liquid.

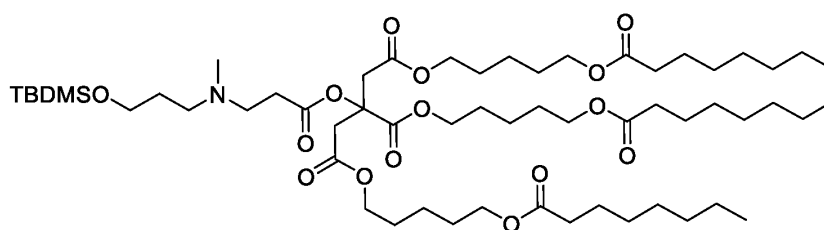
[0771] ¹H-NMR (400 MHz, CDCl₃) - δ 4.21-4.04 (m, 12H), 3.47 (t, *J* = 7.2 Hz, 2H), 3.28 (d, *J* = 15.6 Hz, 2H), 3.20 (d, *J* = 15.6 Hz, 2H), 2.83 (bs, 3H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.30-2.26 (m, 6H), 1.71-1.59 (m, 18H), 1.47-1.39 (m, 15H), 1.36-1.27 (m, 24H), 0.89-0.85 (m, 9H).

Intermediate [X-11]:**X-11**

[0772] Trifluoroacetic acid (4.98 mL, 65.1 mmol) was added into a solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[[(tert-butoxy)carbonyl](methyl)amino]propanoyl]oxy]propane-1,2,3-

- tricarboxylate **[X-10]** (3 g, 2.96 mmol) in dichloromethane (40 mL, 625 mmol) at 0 °C, allow to stirred for until reaction completion. The progress of reaction was monitored by TLC. After completion the reaction, reaction mixture was concentrated under reduced pressure to remove excess trifluoroacetic acid. The reaction mass was quenched with saturated NaHCO₃ solution up to pH 8, and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in Dichloromethane), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[X-11]** (2.85 g, 93 % yield) as pale yellow liquid.
- [0773]** ¹H-NMR (400 MHz, CDCl₃)- δ 4.21-4.16 (m, 2H), 4.10-4.04 (m, 10H), 3.25-3.16 (m, 6H), 2.85-2.81 (m, 3H), 2.72 (s, 3H), 2.28 (t, *J* = 7.6 Hz, 6H), 1.70-1.59 (m, 18H), 1.43 (m, 6H), 1.29-1.27 (m, 24H), 0.87 (t, *J* = 6.8 Hz, 9H).

Intermediate [X-12]:

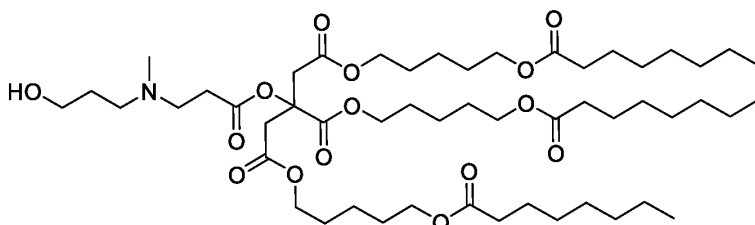


X-12

- [0774]** To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[X-11]** (1.5 g, 1.64 mmol) in acetonitrile (20 mL, 383 mmol), dipotassium carbonate (680 mg, 4.92 mmol), and 2-[(3-bromopropoxy)dimethylsilyl]-2-methylpropan-1-ylum **[X-2]** (623 mg, 2.46 mmol) was added at room temperature, allow to stir at 60 °C for 16 h. The progress of reaction was monitored by TLC and ELSD data. After completion the reaction, reaction mass was cooled, filter by sintered glass funnel. The filtrate was concentrated under reduced pressure and the crude was purified by flash column chromatography (SiO₂: 0-5% Methanol in Dichloromethane), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[(tert-butyl)dimethylsilyl]oxy]propyl](methylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate **[X-12]** (1.0 g, 56 % yield) as pale yellow liquid.

[0775] *ELSD analysis:* Purity 97.32 %, Calculated $C_{58}H_{107}NO_{15}Si = 1085.74$, Observed = 1086.90 (m/z, $M+H^+$).

TL1-12D-011-A (Compound (62))

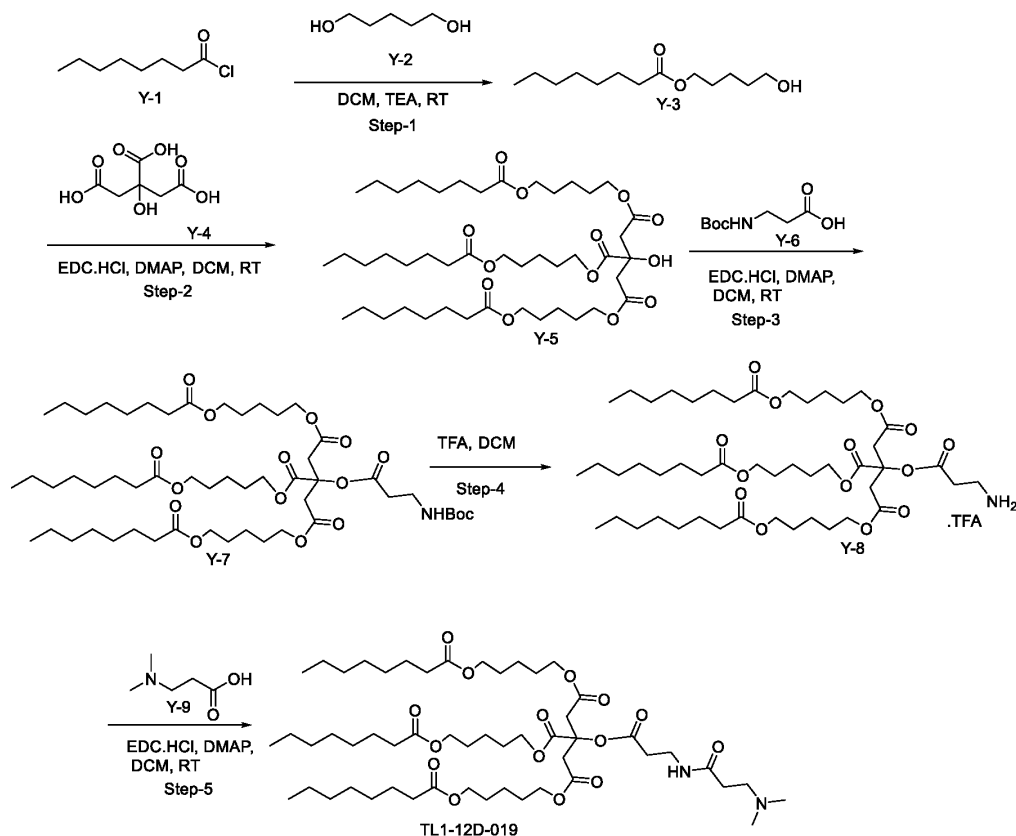


Compound (62)

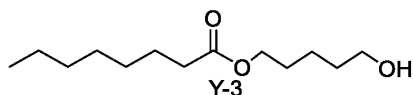
[0776] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[(tert-butyl)dimethylsilyl]oxy]propyl](methyl)amino)propanoyl]oxy}propane-1,2,3-tricarboxylate (0.9 g, 828 μ mol) in tetrahydrofuran (20 mL, 246 mmol), pyridine hydrofluoride (498 μ L, 5.52 mmol) was added at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with cold aqueous sodium bicarbonate solution upto pH 8, and extracted with ethyl acetate (3x50 mL). The resulting organic layer was dried over Na_2SO_4 , concentrated under reduced pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10 % methanol in dichloromethane), to obtain the desired produced [TL1-12D-011-A] (Compound (62)) (0.108 g, 46.56% yield) as a light yellow colour liquid.

[0777] 1H -NMR (400 MHz, $CDCl_3$)- δ 4.16 (t, $J = 6.8$ Hz, 2H), 4.10-4.04 (m, 10H), 3.78 (bs, 1H), 3.29-3.17 (m, 4H), 2.70 (br, 4H), 2.28 (t, $J = 7.6$ Hz, 6H), 1.70-1.59 (m, 18H), 1.55 (bs, 6H), 1.44-1.37 (m, 6H), 1.30-1.25 (m, 26H), 0.87 (t, $J = 6.4$ Hz, 9H).

[0778] *ELSD analysis:* Purity 99.62 %, Calculated $C_{52}H_{93}NO_{15} = 971.65$, Observed = 972.50 (m/z, $M+H^+$).

Example 25. Synthetic Protocol for M1266-527861 (TL1-12D-019) (Compound (17))**Synthetic Scheme Y**

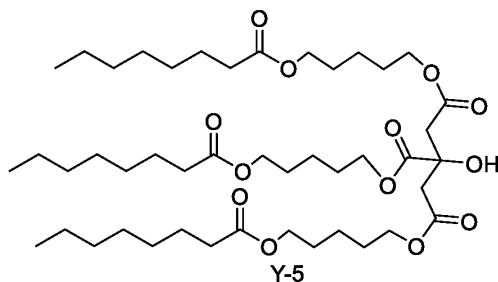
5

Intermediate Y-3

- [0779] To a stirred solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N,N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cooled to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with ethyl acetate (200 ml). Organic layer was washed with brine solution (500.0 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure, get crude. Crude was purified with column chromatography using 5-10% Ethyl acetate in Hexanes, to give compound Y-3 (16 g, 69.5 mmol) as light yellow colour liquid.

[0780] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 6H), 1.44 (m, 2H), 1.29 (s, 8H), 0.87 (t, J = 7.2 Hz, 3H).

Intermediate Y-5



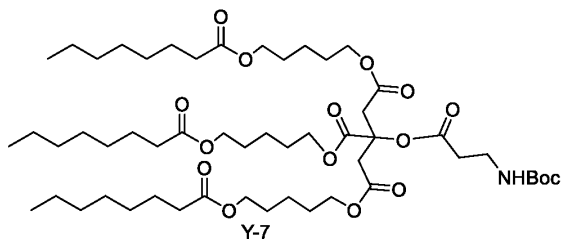
5

[0781] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under inert atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (100.0 mL) and dichloromethane (2x 50.0 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure. Crude was purified with flash chromatography using 10-15% ethyl acetate in hexane to give compound Y-5 (3.5 g, 4.22 mmol) as a light yellow colour liquid.

15

[0782] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 5H), 1.29 (m, 25H), 0.87 (t, J = 7.2 Hz, 9H).

Intermediate Y-7

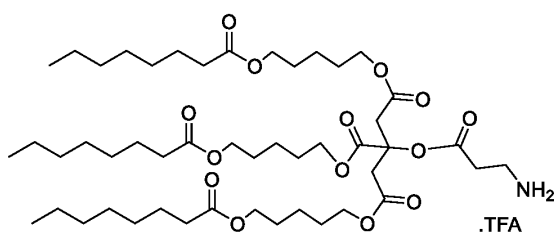


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[0783] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [5] (0.1 g, 121 μmol) in dichloromethane (2.5 mL, 39 mmol) added N,N-

- dimethylpyridin-4-amine (14.7 mg, 121 μ mol) and EDC.HCl (92.5 mg, 4 eq., 482 μ mol) were added at room temperature under nitrogen atmosphere and stirred 30 min at RT, then 4-[[[(tert-butoxy)carbonyl]amino]butanoic acid (98 mg, 4 eq., 482 μ mol) was added in to reaction mass. Reaction mass was stirred over night at RT. Reaction progress was monitored by ELSD. Reaction mixture was diluted with water (50.0 mL) and extracted with DCM (50 ml). Organic layer was washed with brine solution (500.0 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure to get compound Y-7 (0.2 g, 50.5 μ mol) as off white semi solid crude. Which was proceeded to next step without further purification.

Intermediate Y-8



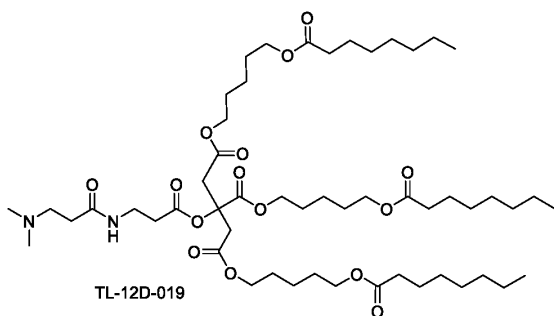
Y-8

10

- [0784] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(4-[(tert-butoxy)carbonyl]amino)butanoyl]oxy propane-1,2,3-tricarboxylate [7] (0.2 g, 1.0eq) in dichloromethane (10 ml) was cooled to 0°C. Added trifluoroacetic acid (0.2 ml , 1v/v) and reaction mass was stirred for 2h at RT. Progress of reaction was monitored by TLC. Once the starting was consumed, solvent was removed under reduced pressure and resulting crude (0.2 g) proceeded for next step without further purification.

15

TL1-12D-019 (Compound (17))



Compound (17)

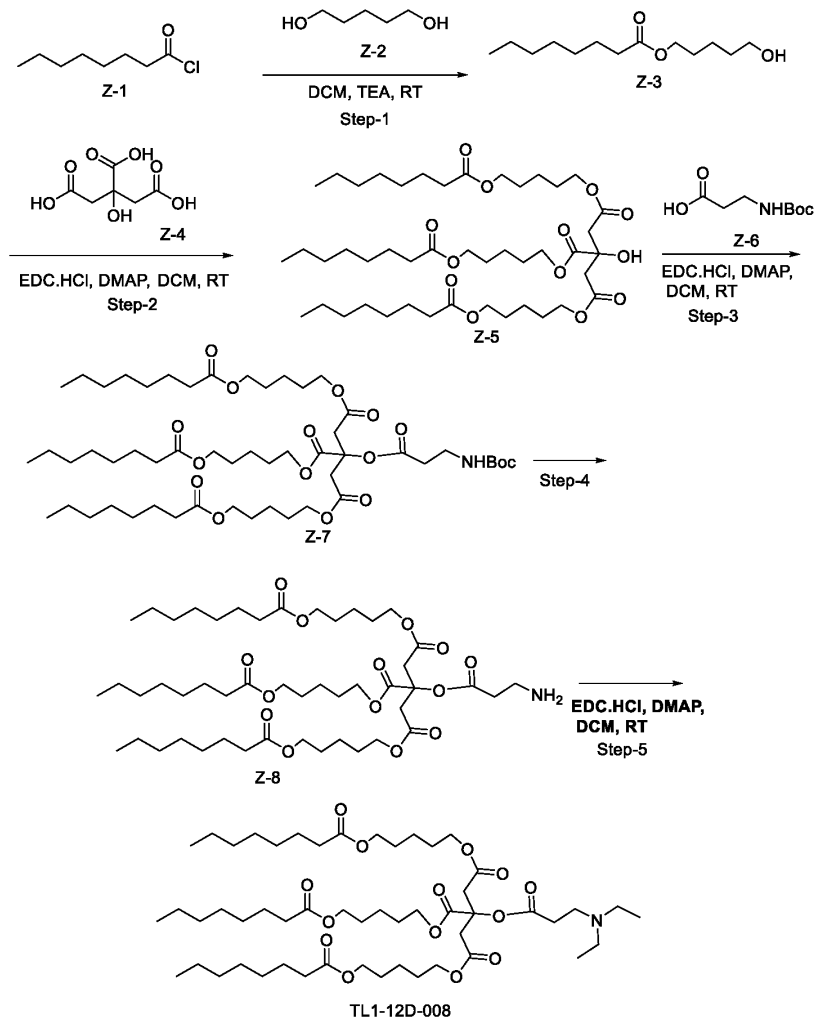
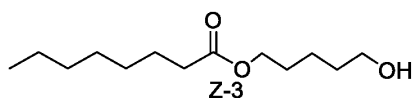
- [0785] To a stirred solution of 3-(dimethylamino)propanoic acid (92.4 mg, 4 eq., 789 μ mol) in dichloromethane (4 mL, 62.5 mmol) was added EDC.HCl (151 mg, 4 eq., 789 μ mol) and N,N-

20

dimethylpyridin-4-amine (24.1 mg, 197 μ mol), reaction mass was stirred 30 min then added 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-aminopropanoyl)oxy]propane-1,2,3-tricarboxylate; trifluoroacetic acid [8] (0.2 g, 197 μ mol). Reaction mass was stirred for 16 h, reaction progress was monitored by ELSD. Starting material was consumed, distil out solvent under reduced pressure to get crude. The resulting crude was purified with Prep ELSD purification and get compound [TL1-12D-019] (Compound (17)) (0.1 g, 0.1 mmol) as a white solid.

[0786] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, J = 6.8 Hz, 2H), 4.074 (m, 10H), 3.29-3.25 (d, J = 15.6 Hz, 2H), 3.21-3.17 (d, J = 15.6 Hz, 2H), 2.44 (bs, 8H), 2.30 (t, J = 7.2 Hz, 6H), 1.65 (m, 22H), 1.40 (m, 6H), 1.27 (brs, 28H), 0.87 (t, J = 2.8 Hz, 9H).

[0787] *LCMS analysis*: Purity 99.66 %, Calculated $\text{C}_{53}\text{H}_{94}\text{N}_2\text{O}_{15}$ = 998.67, Observed = 999.50 (m/z , $\text{M}+\text{H}^+$).

Example 26. Synthetic Protocol for M1266-560527 (TL1-12-008) (Compound (89))**Synthetic Scheme Z****Intermediate Z-3**

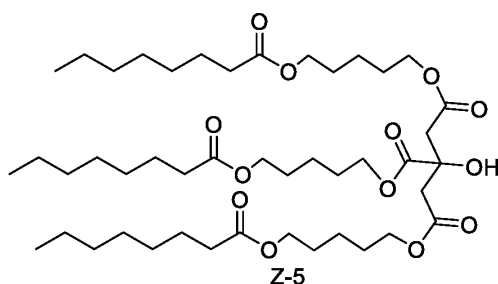
5

- [0788] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N, N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried
- 10

over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxypentyl octanoate [Z-3] (16 g, 69.5 mmol) as light yellow colour liquid.

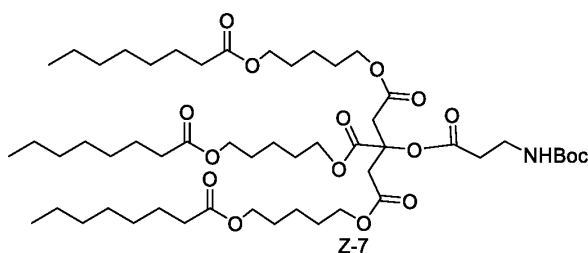
[0789] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H),
 5 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

Intermediate Z-5

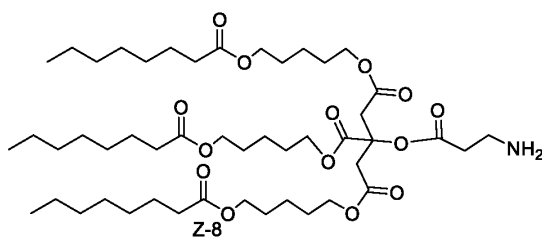


[0790] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [Z-3] (2.5 g, 13 mmol)
 10 in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass
 15 diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [Z-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

[0791] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J =
 20 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (7, J = 7.2 Hz, 9H).

Intermediate Z-7

- [0792] To a stirred solution of 3-[[[(tert-butoxy)carbonyl]amino]propanoic acid [Z-6] (2.28 g, 4 eq., 12.1 mmol) in dichloromethane (50 mL, 781 mmol) was added N,N-dimethylpyridin-4-amine (368 mg, 3.02 mmol) and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (2.6 g, 4.5 eq., 13.6 mmol), then reaction mass was stirred and added 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [Z-5] (2.5 g, 3.02 mmol) at RT. Reaction mass was stirred for 36 h at RT. Reaction progress was monitored by TLC and ELSD. Reaction mass was diluted with DCM (100.0 mL) and washed with water (2x 50.0 mL). Organic layer was separated and dried with sodium sulphate and distil out under reduced pressure.
- [0793] The resulting crude was purified with flash chromatography using 15% ethyl acetate in heptane, to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[[[(tert-butoxy)carbonyl]amino]propanoyl]oxy]propane-1,2,3-tricarboxylate [Int-7] (1.85 g, 1.85 mmol) as a clear liquid.
- [0794] LCMS analysis: Purity 70%, Calculated $C_{53}H_{93}NO_{16} = 999.65$, Observed = 1000.30 (m/z, $M+H^+$).

Intermediate Z-8

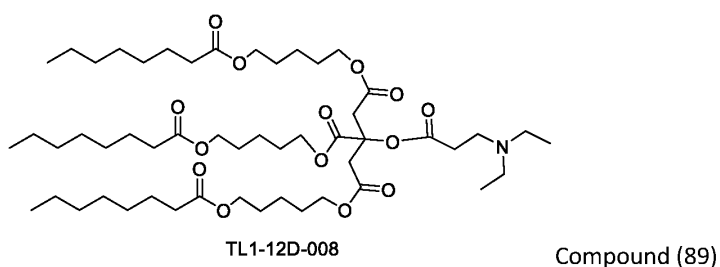
- [0795] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-[[[(tert-butoxy)carbonyl]amino]propanoyl]oxy]propane-1,2,3-tricarboxylate (1.8 g, 1.8 mmol) in DCM, added trifluoroacetic acid (5 mL) at 0°C. Reaction mass was allowed to RT and stirred at RT for 4 h. Reaction progress was monitored by TLC. Reaction mass was distil out under reduced pressure, to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-aminopropanoyl]oxy]propane-1,2,3-tricarboxylate

trifluoroacetic acid (1.83 g, 1.8 mmol) as a colour less semi solid. This compound was used in next step without further purification.

[0796] *LCMS analysis:* Purity 64.94 %, Calculated $C_{48}H_{85}NO_{14}$ = 899.60, Observed = 900.85 (m/z , $M+H^+$).

5

TL1-12D-008 (Compound (89))

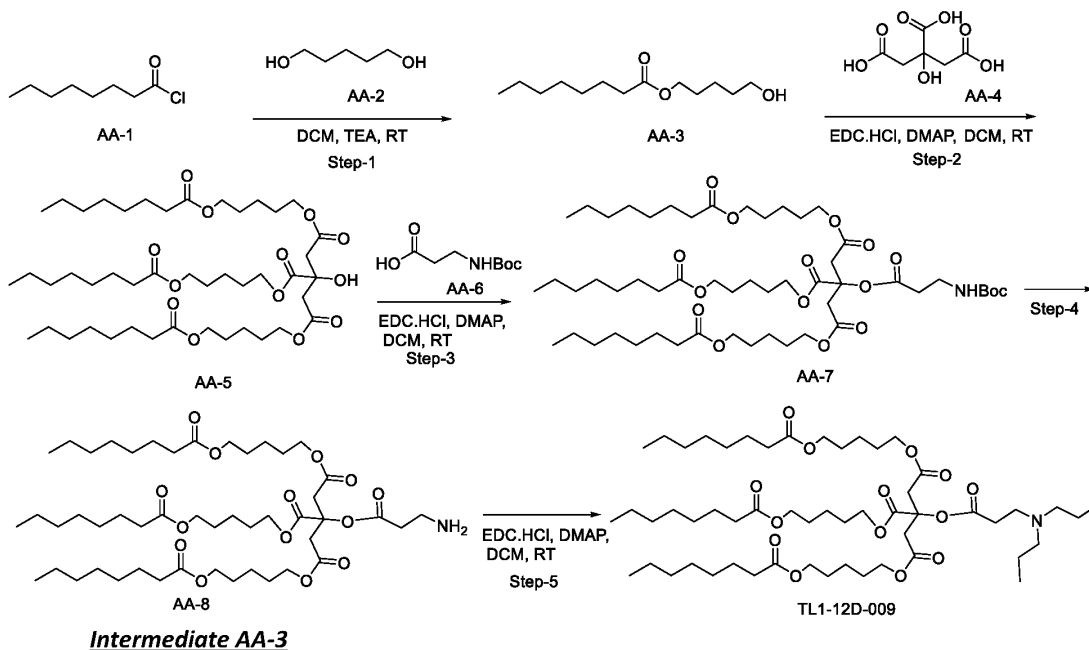


[0797] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-aminopropanoyl)oxy]propane-1,2,3-tricarboxylate hydrochloride [Z-8] (0.5 g, 534 μ mol) in acetonitrile (8 mL, 153 mmol), were added iodoethane (833 mg, 10 eq., 5.34 mmol) and dipotassium carbonate (221 mg, 3 eq., 1.6 mmol). Reaction mass stirred for 48 h at 55°C. Progress of reaction was monitored by ELSD, after consumption of starting material, reaction mass allowed to come at RT and filter through sintered funnel, solid was wash with Ethyl acetate. Filtrate was distilled out under reduced pressure. Crude was purified with prep HPLC, to give [TL1-12D-008] (Compound (89)) (0.1 g, 93.4 μ mol) as a yellow colour liquid.

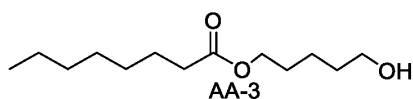
[0798] 1H NMR (400 MHz, DMSO- d_6): δ 4.07 (t, J = 6.4 Hz, 2H), 4.01 (m, 10H), 3.28 (m, 2H), 3.10 (m, 8H), 2.74-2.70 (m, 2H) 2.26 (t, J = 7.2, 6H), 1.61-1.57 (m, 12H), 1.53 (t, J = 6.8 Hz, 6H), 1.35 (m, 6H), 1.24 (brs, 24H), 1.17 (t, J = 7.2 Hz, 6H), 0.85 (t, J = 6.8 Hz, 9H).

[0799] *LCMS analysis:* Purity 99.70 %, Calculated $C_{52}H_{93}NO_{14}$ = 955.66, Observed = 956.45 (m/z , $M+H^+$).

20

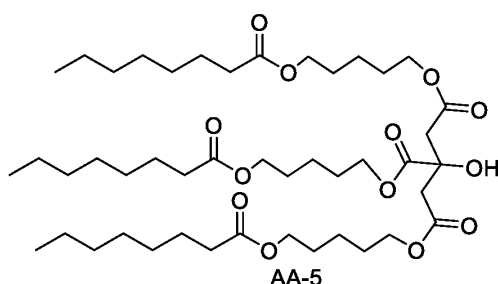
Example 27. Synthetic Protocol for M1266-560531 (TL1-12D-009) (Compound (61))**Synthetic Scheme AA**

5



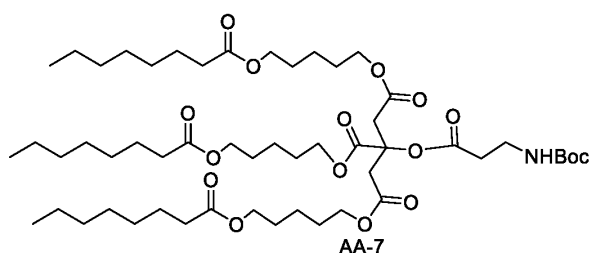
[0800] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N, N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxypentyl octanoate [AA-3] (16 g, 69.5 mmol) as light yellow colour liquid.

[0801] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

Intermediate AA-5

[0802] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [AA-3] (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [AA-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

[0803] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (7, J = 7.2 Hz, 9H).

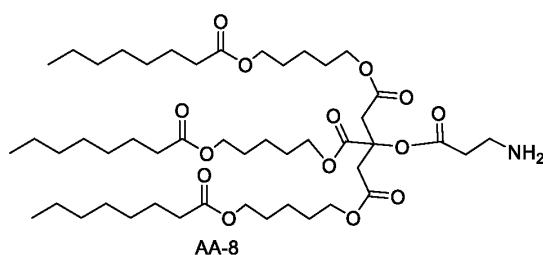
Intermediate AA-7

[0804] To a stirred solution of 3-[[3-[(tert-butoxycarbonyl)amino]propanoate]oxy]propanoic acid [AA-6] (2.28 g, 4 eq., 12.1 mmol) in dichloromethane (50 mL, 781 mmol) was added N,N-dimethylpyridin-4-amine (368 mg, 3.02 mmol) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (2.6 g, 4.5 eq., 13.6 mmol), then reaction mass was stirred and added 1,2,3-tris[5-

(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [Int-5] (2.5 g, 3.02 mmol) at RT. Reaction mass was stirred for 36 h at RT. Reaction progress was monitored by TLC and ELSD. Reaction mass was diluted with DCM (100.0 mL) and washed with water (2x 50.0 mL). Organic layer was separated and dried with sodium sulphate and distil out under reduced pressure. The resulting crude was purified with flash chromatography using 15% ethyl acetate in heptane, to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-[(tert-butoxy)carbonyl]amino)propanoyl]oxy]propane-1,2,3-tricarboxylate [AA-7] (1.85 g, 1.85 mmol) as a clear liquid.

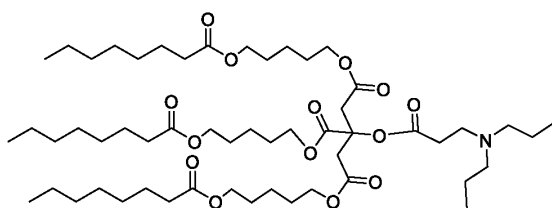
[0805] LCMS analysis: Purity 70%, Calculated $C_{53}H_{93}NO_{16}$ = 999.65, Observed = 1000.30 (m/z, $M+H^+$).

10 **Intermediate AA-8**



[0806] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-[(tert-butoxy)carbonyl]amino)propanoyl]oxy]propane-1,2,3-tricarboxylate [AA-7] (1.8 g, 1.8 mmol) in D7M, added trifluoroacetic acid (5 mL) at 0°C. Reaction mass was allowed to RT and stirred at RT for 4 h. Reaction progress was monitored by TLC. Reaction mass was distil out under reduced pressure to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-aminopropanoyl)oxy]propane-1,2,3-tricarboxylate trifluoroacetic acid (1.83 g, 1.8 mmol) as a colour less semi solid. This compound was used in next step without further purification.

20 **[0807]** LCMS analysis: Purity 64.94 %, Calculated $C_{48}H_{85}NO_{14}$ = 899.60, Observed = 900.85 (m/z, $M+H^+$).

TL1-12D-009 (Compound (61))

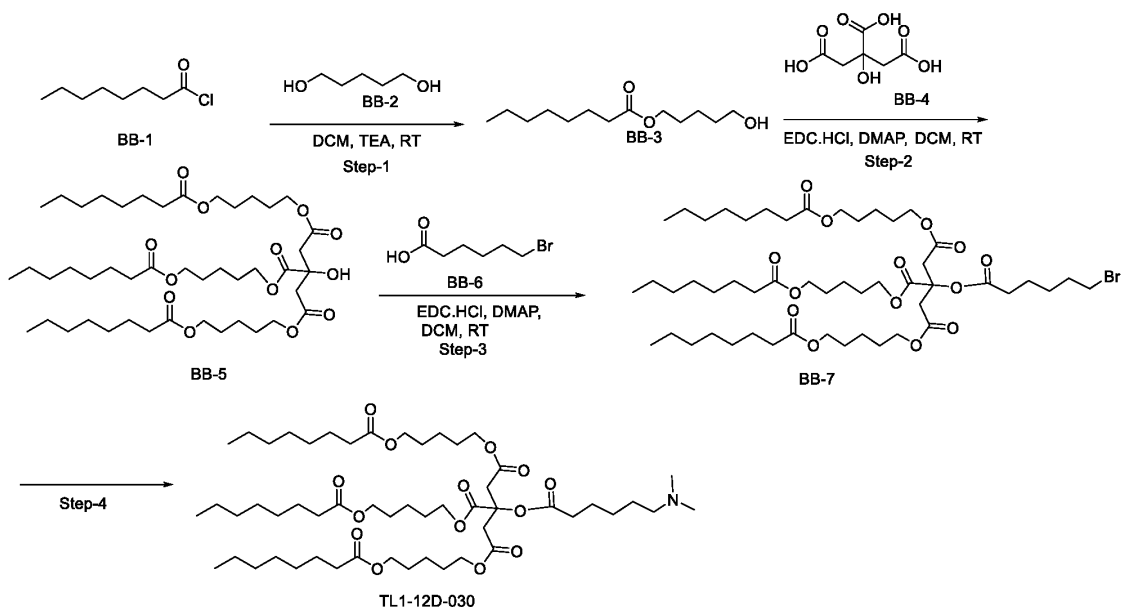
TL1-12D-009

Compound (61)

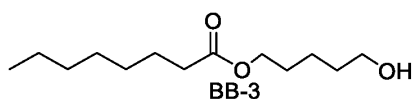
- [0808]** To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-aminopropanoyl)oxy]propane-1,2,3-tricarboxylate hydrochloride [AA-8] (0.5 g, 534 μ mol) in acetonitrile (8 mL, 153 mmol), were added 1-iodopropane (907 mg, 10 eq., 5.34 mmol) and dipotassium carbonate (221 mg, 3 eq., 1.6 mmol). Reaction mass stirred for 48 h at 55°C. Progress of reaction was monitored by ELSD, after consumption of starting material, reaction mass allowed to come at RT and filter through sintered funnel, solid was wash with Ethyl acetate. Filtrate was distilled out under reduced pressure. Crude was purified with prep HPLC, to give [TL1-12D-009] (Compound (61)), TFA salt (62 mg, 56.4 μ mol) as a yellow colour liquid.

[0809] ^1H NMR (400 MHz, DMSO- d_6): δ 4.07 (t, J = 6.0 Hz, 2H), 4.01 (m, 10H), 3.28 (m, 2H), 3.20-3.17 (d, J = 15.2 Hz, 2H), 3.144-3.10 (d, J = 15.6 Hz, 2H), 3.04 (m, 4H), 2.85 (t, J = 7.2 Hz, 2H), 2.26 (t, J = 7.6, 6H), 1.59-1.49 (m, 22H), 1.35 (m, 6H), 1.24 (brs, 24H), 0.85 (t, J = 6.8 Hz, 15H).

- [0810]** LCMS analysis: Purity 99.13 %, Calculated $\text{C}_{54}\text{H}_{97}\text{NO}_{14}$ = 983.69, Observed = 984.65 (m/z, $\text{M}+\text{H}^+$).

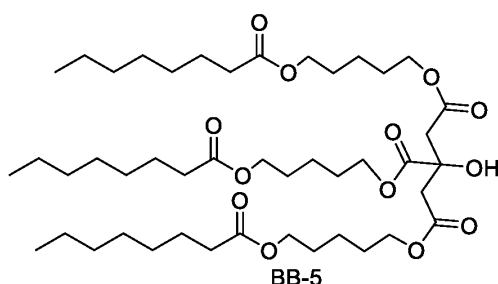
Example 28. Synthetic Protocol for M1266-577919 (TL1-12D-030) (Compound (64))**Synthetic Scheme BB****Intermediate BB-3**

5



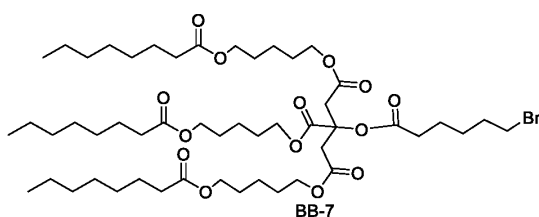
[0811] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N, N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxypentyl octanoate [BB-3] (16 g, 69.5 mmol) as light-yellow colour liquid.

[0812] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

Intermediate BB-5

[0813] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [BB-3] (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [BB-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

[0814] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, $J = 6.4$ Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, $J = 15.6$ Hz, 4H), 2.28 (t, $J = 7.6$ Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (7, $J = 7.2$ Hz, 9H).

Intermediate BB-7

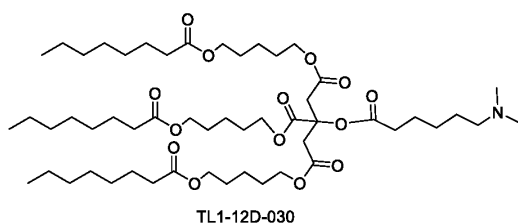
[0815] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate (1.0 g, 1.21 mmol) [BB-5] in dichloromethane (12.5 mL, 195 mmol) was added N,N-dimethylpyridin-4-amine (147 mg, 1.21 mmol) and 6-bromohexanoic acid [BB-6] (941 mg, 4 eq., 4.82 mmol) successively at room temperature and stirred for 10 minute at same temperature, followed by addition of ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine

hydrochloride (925 mg, 4 eq., 4.82 mmol). The resulting reaction mixture was stirred at room temperature for 16 h. Progress of reaction was monitored by TLC / ELSD. The resulting reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (2x30 mL). Combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by flash chromatography on silica gel using 10- 30 % gradient of Ethyl acetate in heptane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(6-bromohexanoyl)oxy]propane-1,2,3-tricarboxylate (160 mg, 159 μmol) as a colourless liquid.

[0816] LCMS analysis: Purity 78%, Calculated C₅₁H₈₉BrO₁₄ = 1004.54, Observed = 1005.30 (m/z, M+H⁺).

10

TL1-12D-030 (Compound (64))

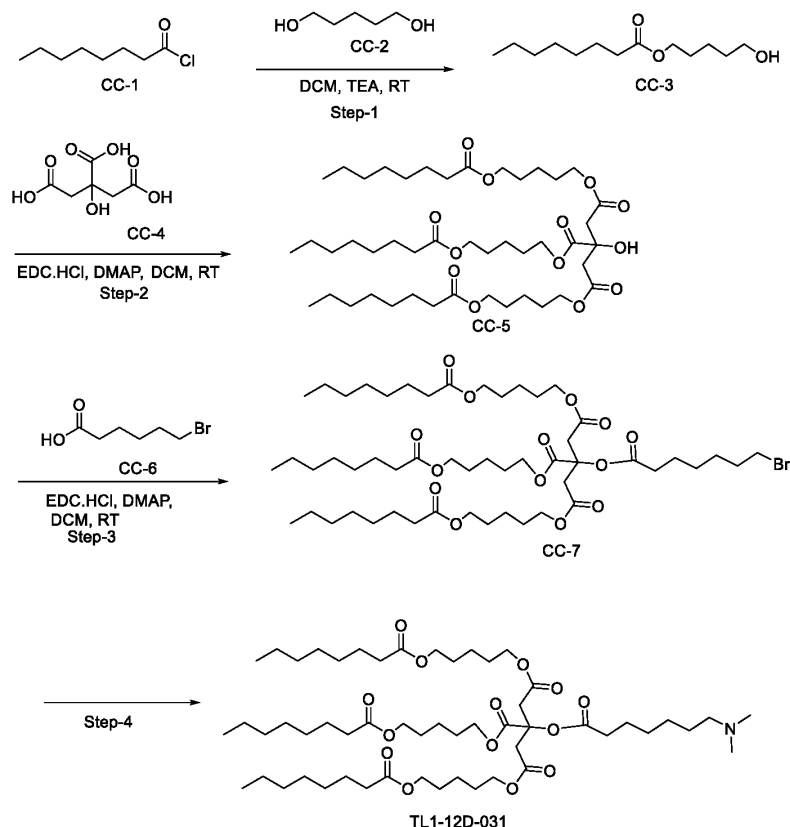


Compound (64)

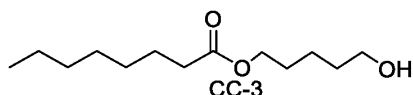
[0817] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(6-bromohexanoyl)oxy]propane-1,2,3-tricarboxylate (1.1 g, 0.57 eq., 1.09 mmol) in tetrahydrofuran (22 mL, 270 mmol), dimethylamine (1.74 g, 20 eq., 38.6 mmol) was successively added at room temperature and stirred for 10 minute at same temperature. The resulting reaction mixture was stirred at 55°C temperature for 16 hrs monitor by TLC. The resulting reaction mixture was quenched with water (50 mL) and extracted with ethyl acetate (2x30 mL). The resulting whole organic were combined and dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by Prep HPLC. The pure fraction were combined and concentrated under reduced pressure to obtain as [TL1-12D-030] (Compound (64)) (260 mg, 268 μmol) a colourless liquid.

[0818] ¹H NMR (400 MHz, CDCl₃): δ 4.05 (t, J = 6.0 Hz, 2H), 4.01 (m, 10H), 3.28-3.26 (d, J = 15.6 Hz, 2H), 3.18-3.14 (d, J = 15.6 Hz, 2H), 2.76-2.75 (d, J = 4.8 Hz, 6H), 2.33 (m, 2H), 2.28 (t, J = 7.2 Hz, 6H), 1.65 (m, 22H), 1.35 (m, 6H), 1.24 (brs, 28H), 0.87 (t, J = 6.8 Hz, 9H).

[0819] LCMS analysis: Purity 97.59 %, Calculated C₅₃H₉₅NO₁₄ = 969.68, Observed = 970.65 (m/z, M+H⁺).

Example 29. Synthetic Protocol for M1266-596266 (TL1-12D-031) (Compound (63))**Synthetic Scheme CC**

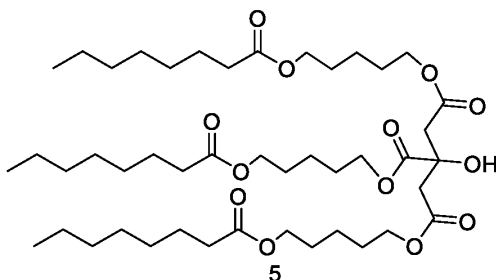
5

Intermediate CC-3

- [0820] To a stirrer solution of pentane-1,5-diol (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N, N-dimethylpyridin-
- 10 4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cool to 0-5°C, then added octanoyl chloride (16 g, 98.4 mmol) dropwise. Reaction mixture was allowed to r.t. and stirred for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with DCM (2x 500.0 mL). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude purified
- 15 with column chromatography using 5-10% ethyl acetate in hexane, to give 5-hydroxypentyl octanoate [CC-3] (16 g, 69.5 mmol) as light-yellow colour liquid.

[0821] ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).

Intermediate CC-5



5

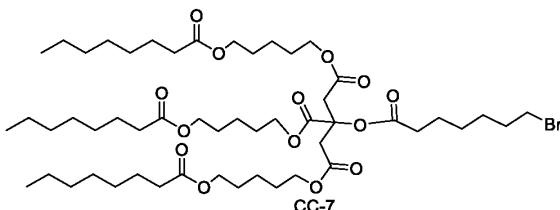
[0822] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [CC-3] (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ([{3-(dimethylamino)propyl}imino)methylidene}(ethyl)amine hydrochloride (9.98 g, 4 eq., 52 mmol). Reaction mass stirred 10 min at RT, then added 5-hydroxypentyl octanoate (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 hr at RT under nitrogen atmosphere. Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (200.0 mL) and dichloromethane (2x 100 mL). Organic layer separated, dried over sodium sulphate, distil out under reduced pressure, get crude. Crude was purified by flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [CC-5] (3.5 g, 4.22 mmol) as a light yellow colour liquid.

15

[0823] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (t, J = 7.2 Hz, 9H).

20

Intermediate CC-7

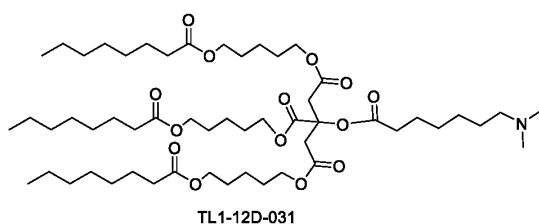


[0824] To a stirred solution 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [CC-5] (1 g, 1.21 mmol) and 7-bromoheptanoic acid (1.01 g, 4 eq., 4.82 mmol) in dichloromethane (20 mL) was added EDC.HCL (925 mg, 4 eq., 4.82 mmol) followed by 4-(dimethylamino)pyridin-1-ium (149 mg, 1.21 mmol). Reaction mass was stirred for 16h at RT.

- 5 Progress of reaction was monitored by ELSD/TLC (SM consumed). Reaction mass was diluted with DCM (25.0 mL) and washed with DM water (50 ml) and dried over sodium sulphate and concentrated. Resulting crude was purified over silica using 10 % ethyl acetate in n-heptane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(7-bromoheptanoyl)oxy]propane-1,2,3-tricarboxylate [Int-7] (0.4 g, 392 μ mol) as yellow gummy mass.

- 10 **[0825]** LCMS analysis: Purity 92.40 %, Calculated $C_{52}H_{91}BrO_{14}$ = 1018.56, Observed = 1020.00 (m/z, $M+H^+$).

TL1-12D-031 (Compound (63))



15

- [0826]** To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(7-bromoheptanoyl)oxy]propane-1,2,3-tricarboxylate (0.8 g, 0.23 eq., 784 μ mol) in tetrahydrofuran (17.8 mL, 218 mmol) and dimethylamine (1.55 g, 10 eq., 34.3 mmol) was successively added at room temperature and stirred for 10 minute at same temperature. The resulting reaction mixture
- 20 was stirred at 55°C temperature for 16 hrs monitor by TLC. The resulting reaction mixture was quenched with water (50 mL) and extracted with ethyl acetate (2x30 mL). The resulting whole organic were combined and dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by Prep HPLC. The pure fraction was combined and concentrated under reduced pressure to obtain the desired product [TL1-12D-031] (Compound
- 25 (63)) as a colourless liquid.

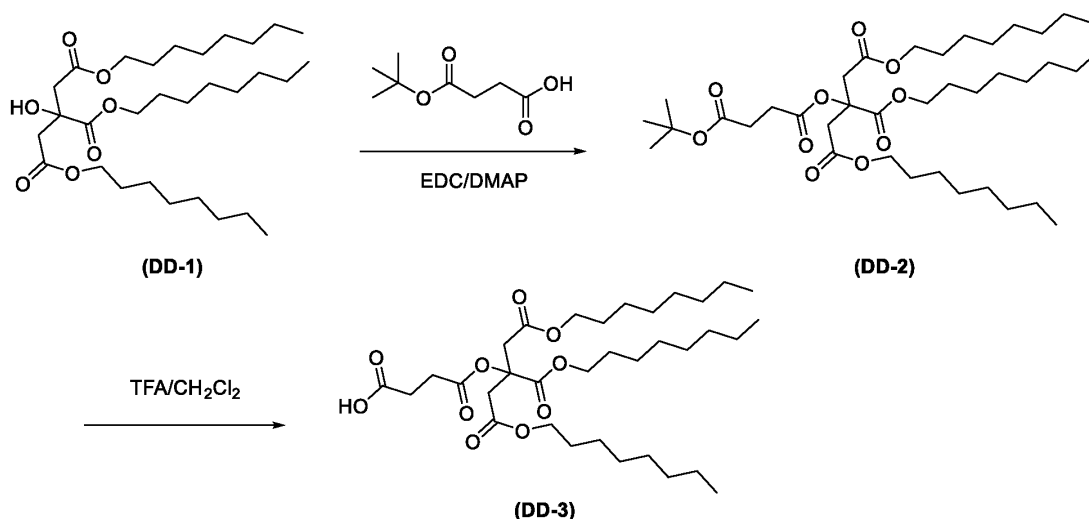
[0827] ¹H NMR (400 MHz, CDCl₃): δ 4.05 (t, J = 6.0 Hz, 2H), 4.01 (m, 10H), 3.18-3.14 (d, J = 15.6 Hz, 2H), 3.08-3.04 (d, J = 15.6 Hz, 2H), 3.02-2.97 (m, 2H), 2.76-2.75 (d, J = 5.2 Hz, 6H) 2.28 (t, J = 7.6 Hz, 8H), 1.55 (m, 22H), 1.35 (m, 6H), 1.24 (brs, 28H), 0.87 (t, J = 6.8 Hz, 9H).

[0828] LCMS analysis: Purity 99.65 %, Calculated $C_{54}H_{97}NO_{14}$ = 983.69, Observed = 984.90 (m/z, $M+H^+$).

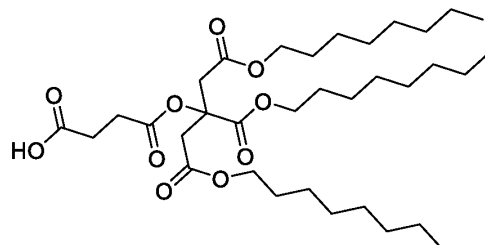
Example 30. Synthesis of Compound (26)

5

Synthetic Scheme DD



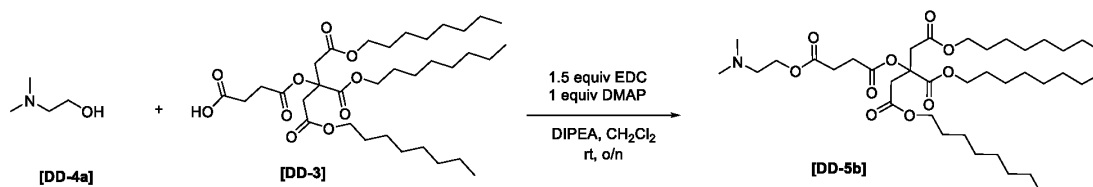
Intermediate (DD-3):



- 10 [0829] Part 1 – To a 100 mL round bottom flask was added tertiary alcohol (DD-1) (2.00 g, 3.78 mmol), mono-tert-butyl succinate (1.98 g, 3.0 equiv), DMAP (462 mg, 1.0 equiv), DIPEA (5.92 mL, 9.0 equiv), and anhydrous CH₂Cl₂ (15 mL). To this stirring mixture at room temperature was added EDC (2.18 g, 3.0 equiv) in one batch. The reaction was stirred at room temperature for 16 h and was monitored by TLC using 20% EtOAc in hexanes. After significant consumption of (DD-1) as
- 15 determined by TLC, the reaction mixture was diluted with CH₂Cl₂ and washed with sat. NaHCO₃ solution, brine, and dried with sodium sulfate. Crude material is then purified using 0-20% EtOAc in hexanes where the desired compound (DD-2) eluded out at 100% hexanes and 10% EtOAc in hexanes. All the fractions containing (DD-2) were combined despite some containing (DD-1) as an

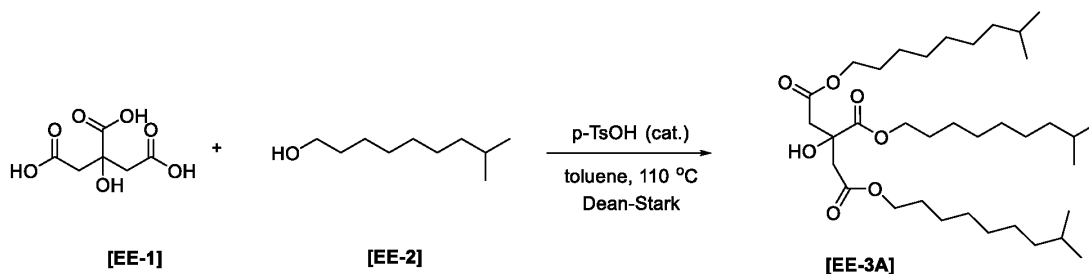
impurity. The combined material was concentrated to dryness to provide intermediate **(DD-2)** as viscous oil.

- [0830]** *Part 2* – To a 50 mL round bottom flask containing the solution of intermediate **(DD-2)** in anhydrous CH_2Cl_2 (3 mL) was added TFA (3 mL) drop wise at room temperature. The reaction was stirred at room temperature until complete consumption of **(DD-2)** as determined by TLC analysis. After 3 h, the reaction was completed, and some toluene was added to the reaction mixture before it was concentrated down to dryness under reduced pressure. The leftover residue was purified using 0-30% EtOAc in hexanes to deliver the desired intermediate **(DD-3)** as viscous colorless oil (2.20 g, 98% over two steps). ^1H NMR (400 MHz, CDCl_3) δ 4.16 – 4.04 (m, 6H), 3.24 (q, J = 15.5 Hz, 4H), 2.66 (s, 4H), 1.67 – 1.56 (m, 6H), 1.33 – 1.23 (m, 30H), 0.90 – 0.85 (m, 9H).



- [0831]** To the scintillation vial, the acid **[DD-3]** (200 mg, 0.318 mmol), dimethyl aminoethanol **[DD-4a]** (85 mg, 0.954 mmol), DMAP (39.0 mg, 0.318 mmol), CH_2Cl_2 (4 mL), and lastly EDC (91.5 mg, 0.477 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of **[DD-3]**. Reaction was diluted with CH_2Cl_2 and washed with NaHCO_3 solution, brine, and dried with sodium sulfate. The crude material was purified using 0-10% MeOH in DCM to afford the product **[DD-5b]** (Compound (26)) as colorless viscous oil (290 mg, 85% yield).

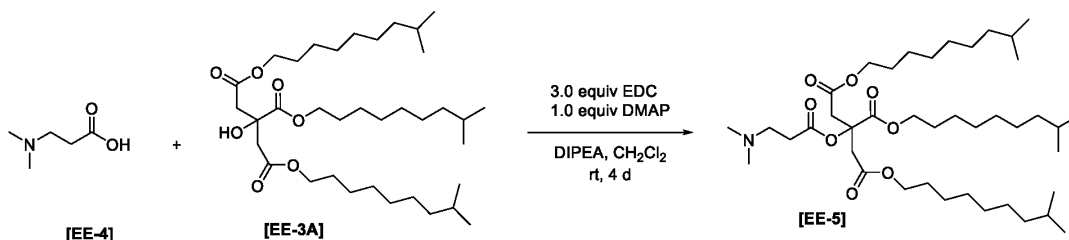
[0832] Analysis: Mass Expected = 700.5 Mass Observed = 700.5

Example 31. Synthesis of Compound (41):**Synthetic Scheme EE-1****Procedure**

- 5 **[0833]** To a 100 mL RBF containing the aliphatic alcohol (2.24 g, 14.2 mmol) was added citric acid (816 mg, 4.25 mmol) and TsOH (269 mg, 1.42 mmol). The solids were then washed down with toluene (10 mL) and heated at 110°C overnight equipped with Dean-Stark apparatus. The remaining residue was slightly diluted with hexanes and loaded onto a large silica gel column. Purification using 0% to 20% EtOAc in hexanes afforded the product **EE-3A** as clear light-yellow oil
- 10 (2.20 g, 85% yield).

Analysis

[0834] $[M+H]^+$ Expected = 613.5 Observed 613.5

Synthetic Scheme EE-215 **Procedure**

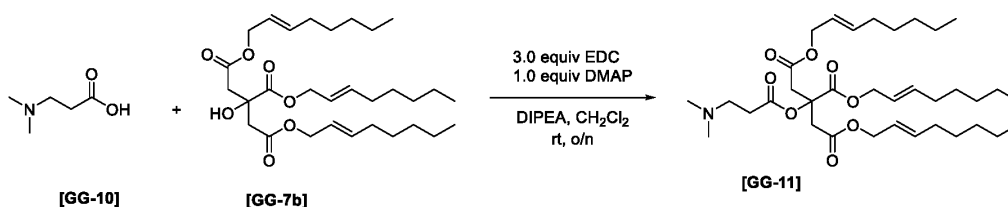
- [0835]** To the scintillation vial, the alcohol **[EE-3A]** (1.0 g, 1.63 mmol), acid **[EE-4]** (752 mg, 4.89 mmol), DMAP (199.0 mg, 1.63 mmol), CH₂Cl₂ (8 mL), DIPEA (2.56 mL, 14.7 mmol), and lastly EDC (938 mg, 4.89 mmol) were added in one batch. The reaction was stirred at room temperature for four days until consumption of **EE-3A**. Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃
- 20 solution, brine, and dried with sodium sulfate. Material was purified using 0%, 20%, and followed by 40% EtOAc in hexanes to elude out product (on TLC plate it takes 80% EtOAc in hexanes to elude). Fractions containing pure product as determined by MS were combined and concentrated. The final product **EE-5** (Compound (41), 420 mg (36%) as a pale yellow and viscous oil.

Analysis

[0836] $[M+H]^+$ Expected = 712.6 Observed 712.6

Example 32. Synthesis of Compound (47)

5

Synthetic Scheme FF**Procedure**

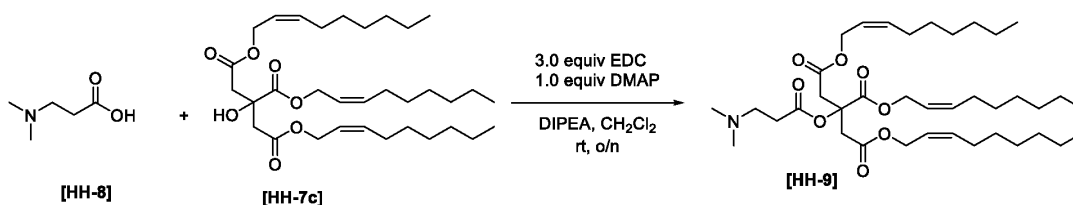
[0837] To the scintillation vial, the alcohol [GG-7b] (724 mg, 1.39 mmol), acid [GG-10] (638 mg, 4.16 mmol), DMAP (169.0 mg, 1.39 mmol), DIPEA (2.17 mL, 12.5 mmol), CH₂Cl₂ (11 mL), and lastly EDC (797 mg, 4.16 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of GG-7b. Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃ solution, brine, and dried with sodium sulfate. Material was purified using 0%, 20%, and followed by 40% EtOAc in hexanes to elude out product (on TLC plate it takes 80% EtOAc in hexanes to elude).

15 Fractions containing pure product as determined by MS were combined and concentrated to dryness to give viscous yellow oil as product GG-11, (Compound (47)), (411 mg, 48% yield).

Analysis

[0838] $[M+H]^+$ Expected = 622.4 Observed 622.5

20

Example 33. Synthesis of Compound (52)**Synthetic Scheme GG**

Procedure

[0839] To the scintillation vial, the alcohol [HH-7c] (829 mg, 1.47 mmol), acid [HH-8] (676 mg, 4.40 mmol), DMAP (179.0 mg, 1.47 mmol), CH₂Cl₂ (12 mL), and lastly EDC (844 mg, 4.40 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of 7c.

- 5 Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃ solution, brine, and dried with sodium sulfate. Material was purified using 0%, 20%, and followed by 40% EtOAc in hexanes to elude out product (on TLC plate it takes 80% EtOAc in hexanes to elude). Fractions containing pure product as determined by MS were combined and concentrated. The final product HH-9 [TL-005] (Compound (52)), 499 mg (51%) as a pale yellow and viscous oil.

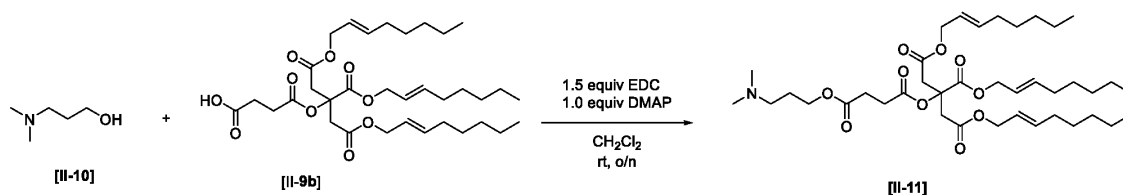
10

Analysis

[0840] [M+H]⁺ Expected = 664.5 Observed 664.5

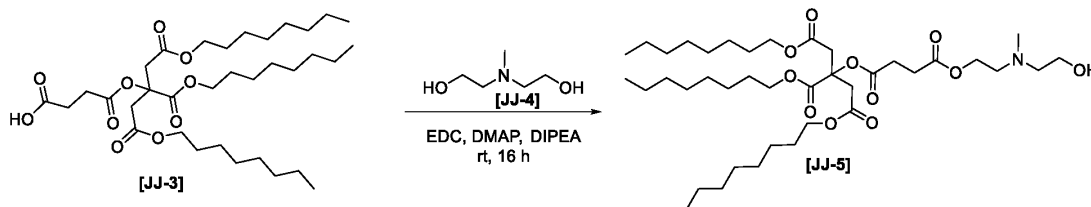
Example 34. Synthesis of Compound (2)

15

Synthetic Scheme HH**Procedure**

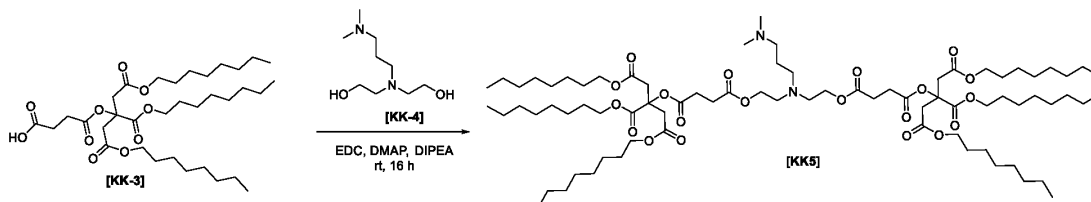
[0841] To the scintillation vial, the acid [II-9b] (250 mg, 0.401 mmol), dimethyl aminopropanol [II-10] (82.8 mg, 0.803 mmol), DMAP (49.0 mg, 0.401 mmol), CH₂Cl₂ (4 mL), and lastly EDC (154 mg, 0.803 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of 9b. Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃ solution, brine, and dried with sodium sulfate. The crude material was purified using 0-30% MeOH in DCM to afford the product II-11 (Compound (2)), 146 mg (51%) as a colorless viscous oil.

- 25 [0842] **Analysis:** Mass expected 708.5 Observed 708.9

Example 35. Synthesis of Compound (4)**Synthetic Scheme II****5 Procedure**

[0843] To the scintillation vial, the acid [3] (300 mg, 0.477 mmol), ethanolamine [JJ-4] (284.24 mg, 2.38 mmol), DMAP (58.24 mg, 0.477 mmol), CH₂Cl₂ (6 mL), DIPEA (0.498 mL, 2.86 mmol), and lastly EDC (137.18 mg, 0.715 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of 3. Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃ solution, brine, and dried with sodium sulfate. The crude material was purified using 5% MeOH in DCM first (10% MeOH/DCM had too high of an RF) and then purified using 5% MeOH in EtOAc to afford the product JJ-5 (Compound (4)), 187 mg (54%) as a colorless viscous oil.

[0844] **Analysis:** Mass expected: 729.50 | Observed: 729.50

15 Example 36. Synthesis of Compound (31)**Synthetic Scheme JJ****Procedure**

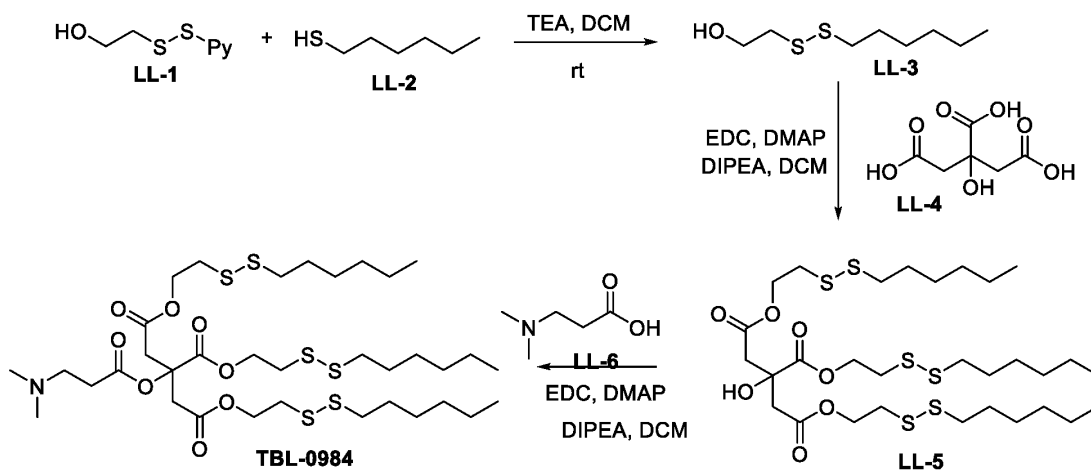
20 [0845] To the scintillation vial, the acid [3] (660 mg, 1.05 mmol), diethanolamine [KK-4] (90.5, 0.475 mmol), DMAP (128.22 mg, 1.05 mmol), CH₂Cl₂ (6 mL), DIPEA (1.09 mL, 6.3 mmol), and lastly EDC (301.8 mg, 1.57 mmol) were added in one batch. The reaction was stirred at room temperature until consumption of SM. Reaction was diluted with CH₂Cl₂ and washed with NaHCO₃ solution, brine, and dried with sodium sulfate. The crude material was purified using 7-10% MeOH in DCM and then re-purified using 0-10% MeOH/EtOAc to result in the product KK-5 [ETH-013]

25 (Compound (31)), 671 mg (45%) as light-yellow viscous oil.

[0846] **Analysis:** Mass expected: 1410.98 | Observed: 1410.92

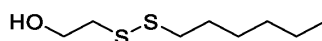
Example 37. Synthesis of Compound (21):

Synthetic Scheme KK



5

2-(hexyldisulfaneyl)ethan-1-ol (LL-3)

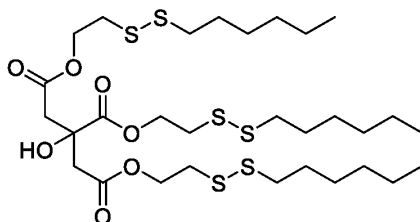


10

[0847] To a solution of LL-1 (1.0 g, 5.34 mmol) and LL-2 (1.26 g, 10.68 mmol) in chloroform was added TEA (2.98 mL, 21.36 mmol). The resulting mixture was stirred at room temperature for 3 h. TLC analysis indicated completion of the reaction (MS: no ionization). The reaction mixture was concentrated, and the crude residue was purified to get the pure product 3 (409 mg, 40%).

15 [0848] *ESI-MS analysis:* Calculated C₈H₁₉OS₂, [M + H]⁺ = 195.09, Observed = No ionization, reaction was monitored by TLC

Tris(2-(hexyldisulfaneyl)ethyl) 2-hydroxypropane-1,2,3-tricarboxylate (LL-5)

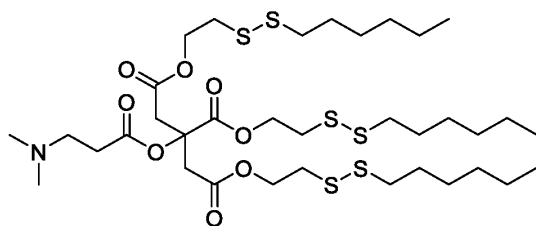


20 [0849] To a solution of citric acid (LL-4) (60 mg, 0.31 mmol) and 2-(hexyldisulfaneyl)ethan-1-ol (LL-3) (200 mg, 1.03 mmol) in 2.5 mL of dichloromethane was added DMAP (38 mg, 0.31 mmol)

and EDC (239 mg, 1.25 mmol), and the resulting mixture was stirred at room temperature for overnight. MS analysis indicated completion of the reaction. Then reaction mixture was diluted with DCM and washed with sat. NaHCO_3 solution, water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified to get tri-ester (LL-5) (140 mg, 62%).

[0850] ESI-MS analysis: Calculated $\text{C}_{30}\text{H}_{60}\text{NO}_7\text{S}_6$, $[\text{M} + \text{NH}_4]^+ = 738.27$, Observed = 738.2

Tris(2-(hexyldisulfaneyl)ethyl) 2-((3-(dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate (Compound (21))



Compound (21))

[0851] To a solution of tri-ester (LL-5) (140 mg, 0.19 mmol) and 3-(dimethylamino)propanoic acid (LL-6) (89 mg, 0.58 mmol) in 3.0 mL of dichloromethane was added DMAP (24 mg, 0.19 mmol) and EDC (112 mg, 0.58 mmol), and the resulting mixture was stirred at room temperature for overnight. MS analysis indicated completion of the reaction. Then reaction mixture was diluted with DCM and washed with sat. NaHCO_3 solution, water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified to get Compound (21) (46 mg, 29%).

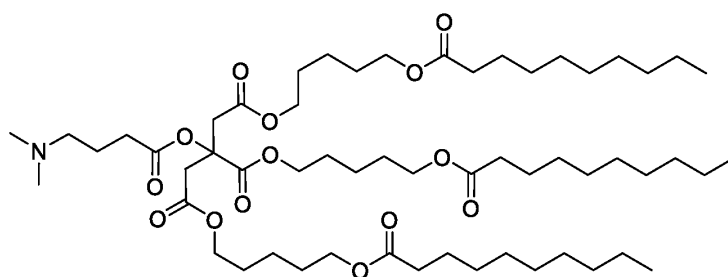
Results:

ESI-MS analysis: Calculated $\text{C}_{35}\text{H}_{66}\text{NO}_8\text{S}_6$, $[\text{M} + \text{H}]^+ = 820.31$, Observed = 820.2

and EDC (1.99 g, 10.41 mmol), and the resulting mixture was stirred at room temperature for overnight. MS analysis indicated completion of the reaction. Then reaction mixture was diluted with DCM and washed with sat. NaHCO_3 solution, water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified to get the intermediate (**MM-5**) (0.66 g, 28%).

[0855] ESI-MS analysis: Calculated $\text{C}_{51}\text{H}_{96}\text{NO}_{13}$, $[\text{M} + \text{NH}_4]^+ = 930.69$, Observed = 930.6

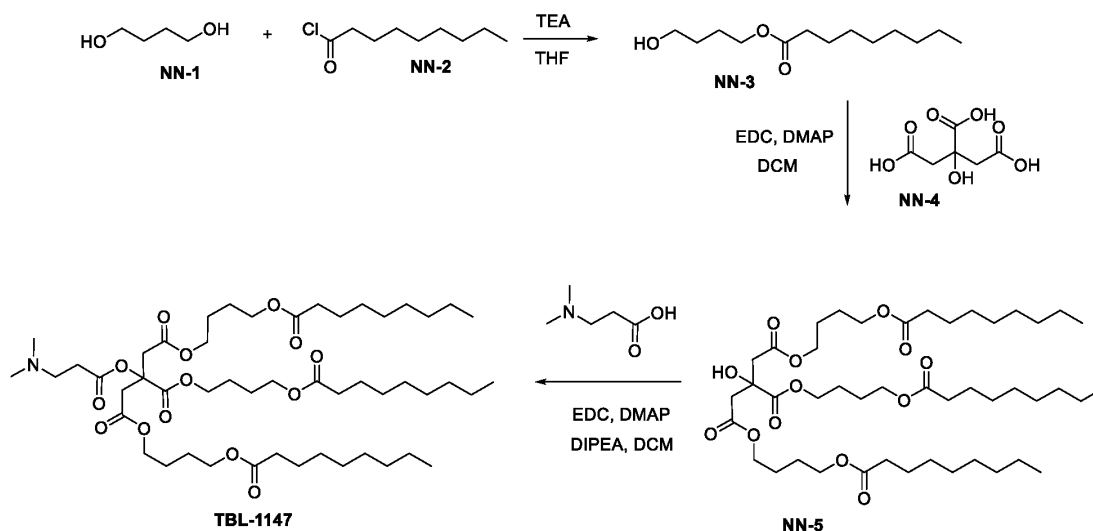
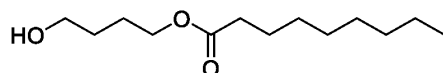
Tris(5-(decanoyloxy)pentyl) 2-((4-(dimethylamino)butanoyl)oxy)propane-1,2,3-tricarboxylate Compound (34)



Compound (34)

[0856] To a solution of intermediate (**MM-5**) (360 mg, 0.39 mmol) and 4-(dimethylamino)butanoic acid (**MM-6**) (198 mg, 1.18 mmol) in 7.0 mL of dichloromethane were added DMAP (48 mg, 0.39 mmol), DIPEA (0.55 mL, 3.15 mmol), and EDC (112 mg, 0.58 mmol), and the resulting mixture was stirred at room temperature for overnight. After 16 h, MS analysis of the reaction mixture indicated ~1:1 ratio of the desired product and starting material **5**. The reaction mixture was diluted with DCM and washed with sat. NaHCO_3 solution, water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified to get the desired product Compound (34) (65 mg, 45%) and recovered 236 mg of intermediate **5**.

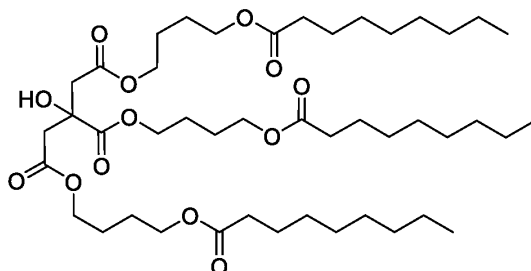
[0857] ESI-MS analysis: Calculated $\text{C}_{57}\text{H}_{104}\text{NO}_{14}$, $[\text{M} + \text{H}]^+ = 1026.75$, Observed = 1026.5

Example 39. Synthesis (Compound (30)):**Synthetic Scheme MM**5 **4-hydroxybutyl nonanoate (NN-3)**

[0858] To a solution of butane-1,4-diol (NN-1) (5.0 mL, 56.59 mmol) in tetrahydrofuran (150
 10 mL) was added nonanoyl chloride (NN-2) (5.1 mL, 28.29 mmol) in tetrahydrofuran (50 mL) at 0 °C. To that was slowly added triethylamine (3.9 mL, 28.29 mmol) and stirred at 0 °C for 2 h. TLC and MS analysis indicated completion of the reaction. The reaction mixture was diluted with dichloromethane washed with sat. NaHCO₃ solution, water and brine. The organic layer was dried over Na₂SO₄ and concentrated. The crude residue was purified to get the desired mono-product
 15 (3.83 g, 58%).

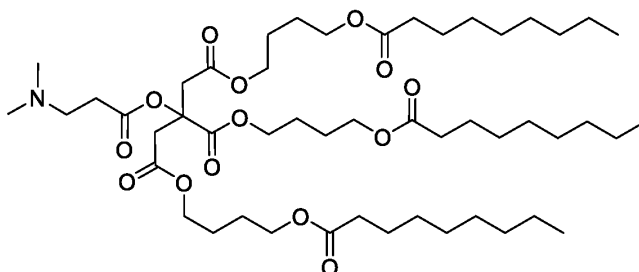
[0859] ESI-MS analysis: Calculated C₁₃H₂₇O₃, [M + H]⁺ = 231.20, Observed = No ionization, reaction was monitored by TLC

Tris(4-(nonanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate (NN-5)



- [0860] To a solution of citric acid (**NN-4**) (0.5 g, 2.60 mmol) and 4-hydroxybutyl nonanoate (**NN-3**) (1.98 g, 8.58 mmol) in 12 mL of dichloromethane was added DMAP (0.32 g, 2.60 mmol) and
- 5 EDC (1.99 g, 10.41 mmol), and the resulting mixture was stirred at room temperature for overnight. MS analysis indicated completion of the reaction. Then reaction mixture was diluted with DCM and washed with sat. NaHCO₃ solution, water and brine solution. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude residue was purified to get the intermediate (**NN-5**) (0.76 g, 35%).
- 10 [0861] ESI-MS analysis: Calculated C₄₅H₈₄NO₁₃, [M + NH₄]⁺ = 846.59, Observed = 846.5

Tris(4-(nonanoyloxy)butyl) 2-((3-(dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate (Compound (30))



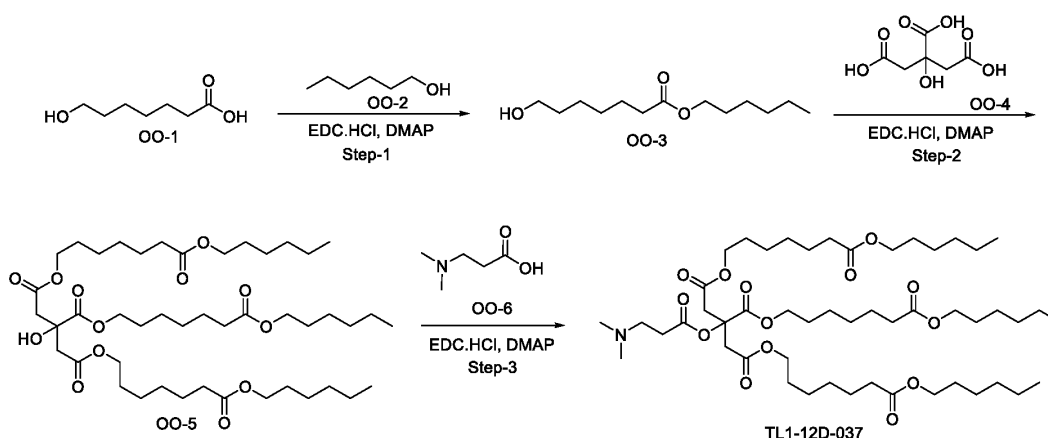
- 15 [0862] To a solution of intermediate (**NN-5**) (760 mg, 0.92 mmol) and 3-(dimethylamino)propanoic acid (**6**) (422 mg, 2.75 mmol) in 15 mL of dichloromethane were added DMAP (112 mg, 0.92 mmol), DIPEA (1.27 mL, 7.33 mmol), and EDC (527 mg, 2.75 mmol), and the resulting mixture was stirred at room temperature for overnight. After 16 h, MS analysis of the
- 20 reaction mixture indicated ~1:1 ratio of the desired product and starting material **5**. The reaction mixture was diluted with DCM and washed with sat. NaHCO₃ solution, water and brine solution. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude residue was

purified to get the desired product (Compound (30)) (46 mg, 13%) and recovered 450 mg of intermediate 5.

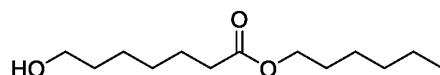
[0863] ESI-MS analysis: Calculated $C_{50}H_{90}NO_{14}$, $[M + H]^+ = 928.64$, Observed = 928.5

5 **Example 40. Synthesis of TL1-12D-037 (Compound (90)):**

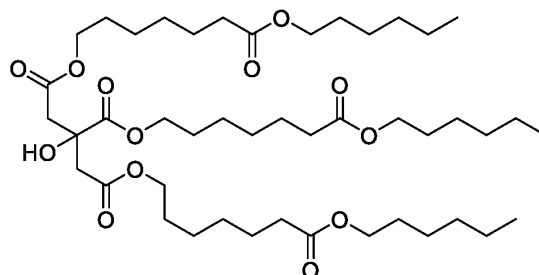
Synthetic Scheme NN



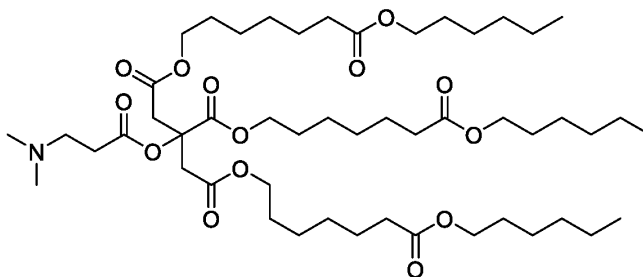
Intermediate [OO-3]:



- 10 [0864] To a stirred solution of 7-hydroxyheptanoic acid [OO-1] (4.5 g, 30.8 mmol) and hexan-1-ol [OO-2] (15.7 g, 5 eq., 0.154 mol) in dichloromethane (90 ml) was added {3-[cyano(ethyl)amino] propyl}dimethylazanium chloride (8.85 g, 1.5 eq., 46.2 mmol) followed by addition of N,N-dimethylpyridin-4-amine (3.76 g, 1.0 eq., 30.8 mmol) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 16h. The reaction was diluted with
- 15 water (100 mL), its organic layer was separated, and aqueous layer was extracted with dichloromethane 2×100 mL. Combined organic layer was washed with brine solution (100 mL), dried over sodium sulphate, and concentrated under reduced pressure. The resulting crude was purified over silica using 5-10% ethyl acetate in n-heptane as eluent system to give product hexyl 7-hydroxyheptanoate [OO-3] (2.1 g, Yield – 29.62%) as a colourless liquid.
- 20 [0865] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- 4.05 (t, $J = 6.4$ Hz, 2H), 3.66-3.61 (dd, $J = 6.4$ Hz, 2H), 2.30 (t, $J = 7.6$ Hz, 2H), 1.67-1.54 (m, 6H), 1.37-1.20 (m, 10H), 0.89 (t, $J = 7.2$ Hz, 3H).

Intermediate [OO-5]:

- [0866]** To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [OO-4] (4 g, 20.8 mmol) in dichloromethane (300 mL) was added {3-[cyano(ethyl)amino]propyl}dimethylazanium chloride (16 g, 4 eq., 83.3 mmol) followed by addition of N,N-dimethylpyridin-4-amine (5.09 g, 2 eq., 41.6 mmol) under nitrogen atmosphere. The reaction mass was stirred for 15 min at room temperature and Hexyl 7-hydroxyheptanoate [OO-3] (16.8 g, 3.5 eq., 72.9 mmol) was added in to reaction mixture. Reaction mixture was stirred for 48 h at room temperature. The resulting reaction mixture was quenched with water (500 mL), organic layer was separated, and aqueous layer was extracted with dichloromethane 2×100 mL. The resulting organic layer was combined, dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by silica using 10-20% ethyl acetate in n-heptane as eluent system. The pure fractions were combined and concentrated under reduced pressure to obtain as tri[6-(hexyloxycarbonyl)hexyl] 2-hydroxy-1,2,3-propanetricarboxylate [OO-5] (3.5 g, Yield – 20.34%).
- [0867]** ¹H-NMR (400 MHz, CDCl₃)- 4.19 (t, *J* = 6.8 Hz, 2H), 4.08-4.03 (m, 10H), 2.89-2.76 (dd, *J* = 15.6 Hz, 4H), 2.28 (t, *J* = 7.2 Hz, 6H), 1.69-1.57 (m, 18H), 1.36-1.27 (m, 30H), 0.88 (t, *J* = 7.2 Hz, 9H). ELSD analysis: Purity 99.63%, Calculated: C₄₅H₈₀O₁₃= 828.55, Observed = 829.50 (*m/z*, M+H⁺).

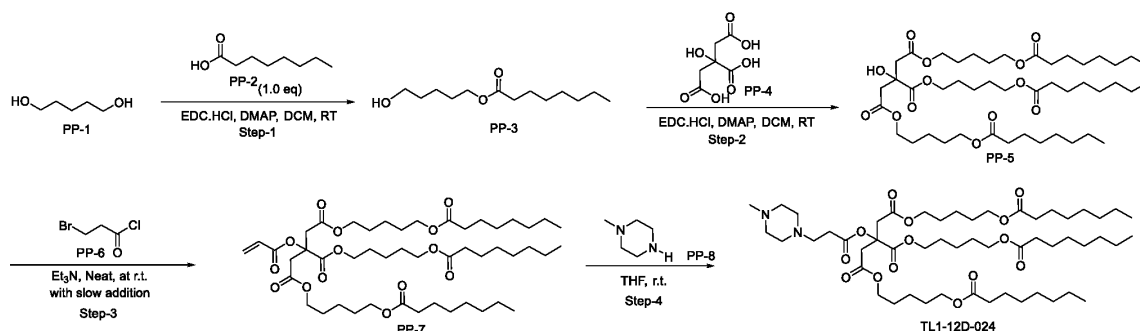
TI1-12D-037 (Compound (90)):

- [0868] To a stirred solution of 3-(dimethylamino) propanoic acid [OO-6] (1.58 g, 4 eq., 13.5 mmol), in dichloromethane (30 mL, 469 mmol) was successively added {3-[cyano(ethyl)amino] propyl}dimethylazanium chloride (2.59 g, 4 eq., 13.5 mmol) followed by addition of 4-(dimethylamino)pyridin-1-ium (832 mg, 2 eq., 6.75 mmol) at room temperature. Reaction mixture
- 5 was stirred for 15 minute at room temperature and 1,2,3-tris[7-(hexyloxy)-7-oxoheptyl] 2-hydroxypropane-1,2,3-tricarboxylate [5] (2.8 g, 3.38 mmol) was added in to reaction mixture. The resulting reaction mixture was stirred at room temperature for 16 h. The resulting reaction mixture was quenched with water 100 mL and extracted with dichloromethane 2 × 30 mL. The resulting organic layer was combined and dried over Na₂SO₄ and concentrated under reduced
- 10 pressure. The resulting crude material was purified by silica using 40-50% ethyl acetate in n-heptane as eluent system. The pure fractions were combined and concentrated under reduced pressure to obtain as 1,2,3-tris[7-(hexyloxy)-7-oxoheptyl] 2-[[3-(dimethylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate [TL1-12D-037] (2.2 g, 2.37 mmol, Yield – 70.18 %) a light brown liquid.
- 15 [0869] ¹H-NMR (400 MHz, DMSO-*d*₆): 4.04-3.97 (m, 12H), 3.17-3.02 (dd, *J* = 15.2 Hz, 4H), 2.45-2.39 (dd, *J* = 6.0 Hz, 4H), 2.26 (t, *J* = 7.6 Hz, 6H), 2.10 (s, 6H), 1.55-1.47 (m, 18H), 1.269-1.261 (br d, *J* = 3.2 Hz, 30H), 0.85 (t, *J* = 6.8 Hz, 9H). ELSD analysis: Purity 99.47 %, Calculated C₅₀H₈₉NO₁₄ = 927.62, Observed = 928.50 (m/z, M+H⁺).

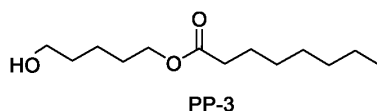
Example 40A. Synthesis of TL1-12D-024 (Compound 100):

20

Synthetic Scheme OO



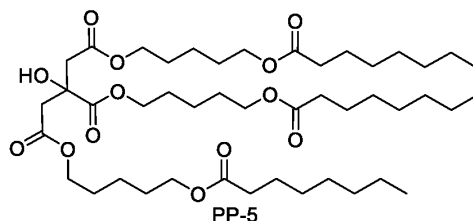
Intermediate [PP-3]:



- [0870] To a stirred solution of octanoic acid [PP-2] (100.0 g, 693 mmol) and pentane-1,5-diol [PP-1] (65.0 g, 624 mmol) in dichloromethane (2000 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (167.0 g, 874 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (17.1 g, 139 mmol). The reaction mixture was stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture and extracted with DCM (3x500 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl octanoate [PP-3] (60.0 g, 37.56% Yield) as a yellow liquid.

¹H-NMR (400 MHz, CDCl₃)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 5H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

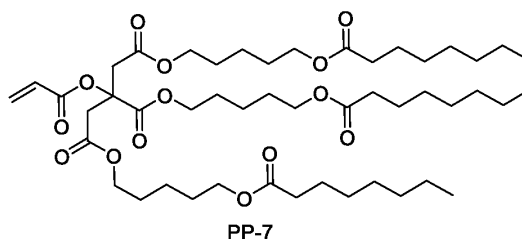
Intermediate [PP-5]:



- [0871] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [PP-4] (12.5 g, 65.1 mmol) and 5-hydroxypentyl octanoate [PP-3] (60.0 g, 260 mmol) in dichloromethane (500 mL) was cooled to 0 °C, ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (49.9 g, 260 mmol) was added followed by 4-(dimethylamino)pyridine-1-ium (8.02 g, 65.1 mmol). The reaction mixture was stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes) to give the desired tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [PP-5] (21.0 g, yield 38.9%) as a yellow liquid.

[0872] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H). ELSD analysis: Purity 97.90 %, Calculated $\text{C}_{45}\text{H}_{80}\text{O}_{13}$ = 828.56, Observed = 829.35 (m/z , $\text{M}+\text{H}^+$).

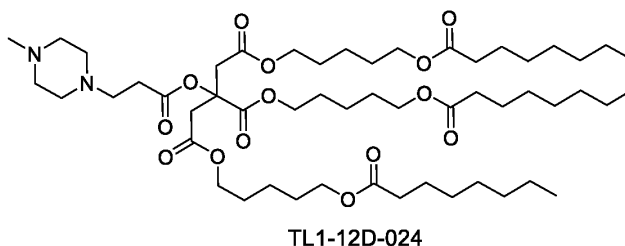
5 **Intermediate [PP-7]:**



[0873] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [PP-5] (10 g, 12.1 mmol) in triethylamine (6.10 g, 60.3 mmol) added 3-bromopropanoyl chloride (1.21 mL, 12.1 mmol) was successively at 0 °C and stirred for 16 h, at same 25 °C temperature. The progress of reaction was monitored by ELSD/TLC (SM was consumed). The reaction mass was diluted with ethyl acetate, filtered through celite and washed with ethyl acetate to get crude product. The crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired [PP-7] (2.0 g, 18.7% yield) as yellow liquid.

15 **[0874]** $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 6.42 (d, J = 17.2 Hz, 1H), 6.12-6.05 (m, 1H), 5.89 (d, J = 10.4 Hz, 1H), 4.17 (t, J = 6.4 Hz, 2 H), 4.09-4.03 (m, 10H), 3.34 (d, J = 15.2 Hz, 2H), 3.25 (d, J = 15.6Hz, 2H), 2.28 (t, J = 7.2 Hz, 6 H), 1.70-1.59 (m, 18H), 1.43-1.35 (m, 6H), 1.28 (s, 24H), 0.87 (t, J = 6.8 Hz, 9 H). ELSD analysis: Purity 99.84%, Calculated $\text{C}_{48}\text{H}_{82}\text{O}_{14}$ = 882.57, Observed = 883.40 (m/z , $\text{M}+\text{H}^+$).

TL1-12D-024 (Compound 100):



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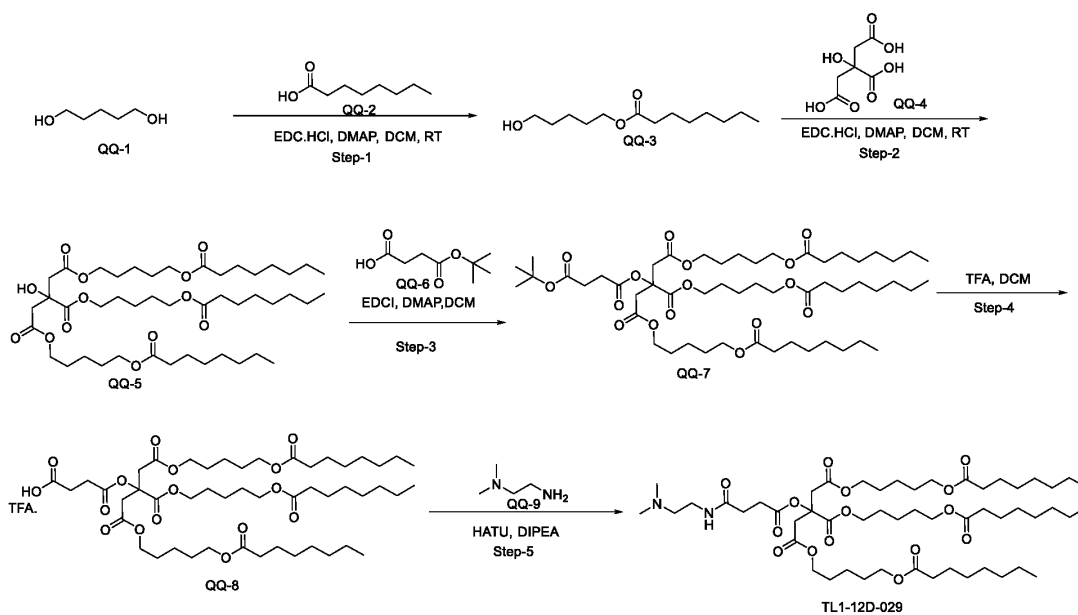
[0875] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate [PP-7] (140 mg, 159 μmol) in tetrahydrofuran (5 mL, 61.4 mmol) was added 1-

5 methylpiperazine [PP-8] (15.9 mg, 159 μ mol). Reaction mass was stirred at RT for 48 h. Progress of the reaction was monitored by TLC. Reaction mass was distilled out under reduced pressure and resulting crude material was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexanes), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(4-methylpiperazin-1-yl)propanoyl]oxy]propane-1,2,3-tricarboxylate [TL1-12D-024] (0.1 g, yield 64.15%) as yellow liquid.

[0876] ¹H-NMR (400 MHz, CDCl₃)- δ 4.15 (t, J = 6.4 Hz, 2H), 4.09-4.04 (m, 10H), 3.27 (d, J = 16.0 Hz, 2H), 3.22 (d, J = 15.6 Hz, 2H), 2.70-2.66 (m, 2H), 2.55-2.48 (m, 6H), 2.35 (s, 2H), 2.28 (t, J = 7.6 Hz, 6H), 2.04 (bs, 5H), 1.69-1.57 (m, 18H), 1.44-1.37 (m, 6H), 1.32-1.27 (m, 24H), 0.87 (t, J = 6.8 Hz, 9H). ELSD analysis: Purity 99.32 %, Calculated C₅₃H₉₄N₂O₁₄ = 982.67, Observed = 983.65 (m/z, M+H⁺).

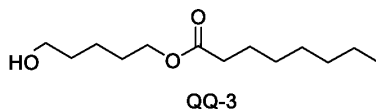
Example 40B: Synthesis of of (TL1-12D-029) (Compound 101):

Synthetic Scheme PP



15

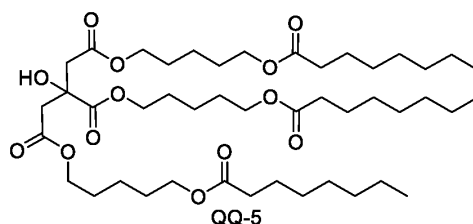
Intermediate [QQ-3]:



[0877] To a stirred solution of octanoic acid [QQ-2] (10 g, 69.3 mmol) and pentane-1,5-diol [QQ-1] (6.5 g, 62.4 mmol) in dichloromethane (200 mL) was cooled to 0 °C and ([3-(dimethylamino) propyl]imino)methylidene(ethyl)amine hydrochloride (16.7 g, 87.4 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.71 g, 13.9 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture, and extract with DCM (3x500 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl octanoate [QQ-3] (6.0 g, 26.0 mmol, yield 37.56%) as pale-yellow liquid.

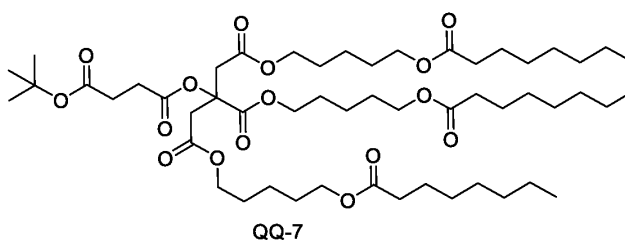
[0878] ¹H-NMR (400 MHz, CDCl₃)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 4H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

Intermediate [QQ-5]:



[0879] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [QQ-4] (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate [QQ-3] (6 g, 26 mmol) in dichloromethane (50 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired compound tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [QQ-5] (2.1 g, yield 38.9%) as a yellow liquid.

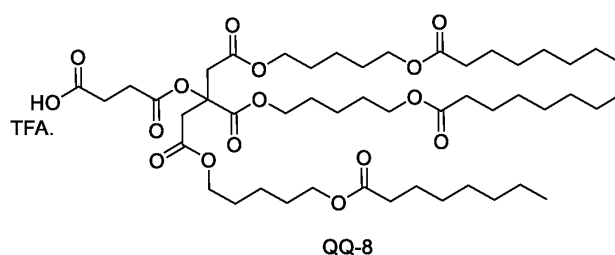
[0880] ¹H-NMR (400 MHz, CDCl₃)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, *J* = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H). ELSD analysis: Purity 97.90 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.35 (*m/z*, M+H⁺).

Intermediate [QQ-7]:

[0881] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [QQ-5] (1.19 g, 1.44 mmol) and 4-(tert-butoxy)-4-oxobutanoic acid [QQ-6] (1 g, 5.74 mmol) in dichloromethane (50 mL) was cooled to 0 °C and EDC. HCl (1.1 g, 5.74 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (177 mg, 1.44 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x50 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give the desired 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[4-(tert-butoxy)-4-oxobutanoyl]oxy]propane-1,2,3-tricarboxylate [QQ-7] (1.0 g, 1.01 mmol, yield 70.72 %) as a pale yellow liquid.

[0882] *ELSD analysis:* Purity 99.59 %, Calculated C₅₃H₉₂O₁₆ = 984.64, Observed = 1002.65 (m/z, M+NH₄⁺).

15 **Intermediate [QQ-8]:**

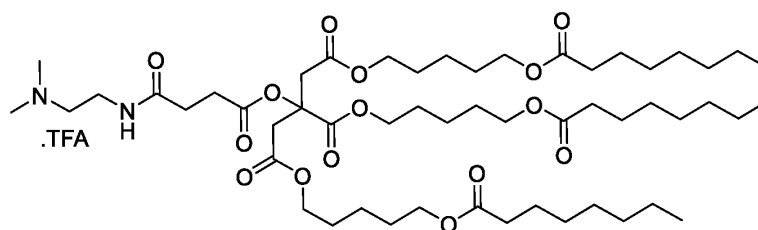


[0883] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[4-(tert-butoxy)-4-oxobutanoyl]oxy] propane-1,2,3-tricarboxylate [QQ-7] (0.8 g, 812 μmol) in dichloromethane (25 mL, 390 mmol) was added trifluoroacetic acid (741 mg, 6.5 mmol) at 0 °C. The resulting reaction mixture was stirred for 16 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with triethylamine up to pH 7. The resulting organic layer concentrated under reduce pressure to give

4-((18-(((5-(octanoyloxy)pentyl)oxy)carbonyl)-8,16,20,28-tetraoxo-9,15,21,27-tetraoxapentatriacontan-18-yl)oxy)-4-oxobutanoic acid 2,2,2-trifluoroacetic acid [QQ-8] (700 mg, crude), which was used in next step as such.

[0884] *ELSD analysis:* Purity 94.19 %, Calculated $C_{49}H_{84}O_{16}$ = 928.58, Observed = 951.55 (m/z, M+Na⁺).

TL1-12D-029 (Compound 101):

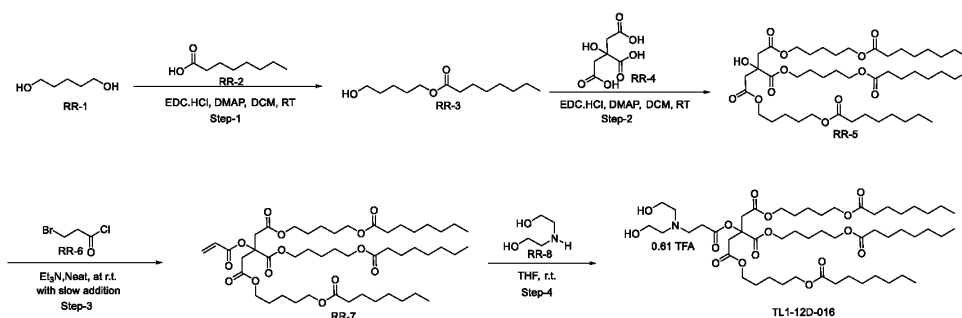
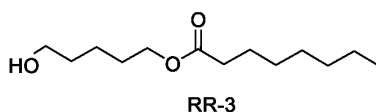


TL1-12D-029

[0885] To a stirred solution of 4-[[1,5-bis{[5-(octanoyloxy)pentyl]oxy}-3-[[5-(octanoyloxy)pentyl]oxy]carbonyl]-1,5-dioxopentan-3-yl]oxy]-4-oxobutanoic acid [QQ-8] (651 mg, 0.7 mmol) and (2-aminoethyl)dimethylamine (80.3 mg, 911 μ mol) [QQ-9] in dichloromethane (15 mL, 234 mmol) was cooled to 0 °C and [bis(dimethylamino)methylidene]({3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl})oxidanium (247 mg, 1.05 mmol) was added followed by ethylbis(propan-2-yl)amine (272 mg, 2.1 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). The reaction mixture was concentrated and resulting crude material was purified by Prep HPLC. The pure fraction were combined and concentrated under reduced pressure to obtained 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-[[2-(dimethylamino)ethyl]carbonyl]propanoyl)oxy]propane-1,2,3-tricarboxylate 2,2,2-trifluoroacetic acid [TL1-12D-029] (0.3 g, yield 42.86 %) as a Light yellow liquid.

[0886] ¹H-NMR (400 MHz, CDCl₃)- δ 4.12 (t, *J* = 6.4 Hz, 2H), 4.09-4.03 (m, 10H), 3.64 (bs, 2H), 3.27-3.17 (m, 6H), 2.86 (s, 6H), 2.65 (t, *J* = 6.4 Hz, 2H), 2.45 (t, *J* = 6.4 Hz, 2H), 2.28 (t, *J* = 7.6 Hz, 6H), 1.70-1.58 (m, 18H), 1.44 (m, 6H), 1.31-1.28 (m, 24H), 0.87 (t, *J* = 6.8 Hz, 9H). *ELSD analysis:* Purity 99.39 %, Calculated $C_{53}H_{94}N_2O_{15}$ = 998.67, Observed = 999.65 (m/z, M+H⁺).

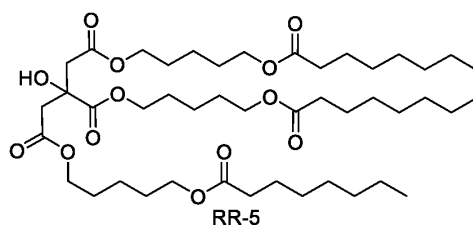
Example 40C. Synthesis of TL1-12D-016 (Compound 105):

**Intermediate [RR-3]:**

- [0887] To a stirred solution of octanoic acid [RR-2] (100.0 g, 693 mmol) and pentane-1,5-diol [RR-1] (65.0 g, 624 mmol) in dichloromethane (2000 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (167.0 g, 874 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (17.1 g, 139 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture, and extract with DCM (3x500 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give 5-hydroxypentyl octanoate [RR-3] (60.0 g, yield 37.56% Yield) as yellow liquid.

[0888] ¹H-NMR (400 MHz, CDCl₃)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 5H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

- 15 Intermediate [RR-5]:

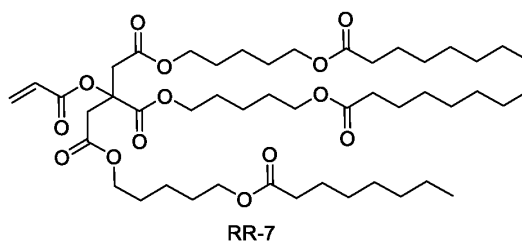


[0889] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [RR-4] (12.5 g, 65.1 mmol) and 5-hydroxypentyl octanoate [RR-3] (60.0 g, 260 mmol) in dichloromethane (500 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine

hydrochloride (49.9 g, 260 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (8.02 g, 65.1 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under
5 reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [RR-5] (21.0 g, 38.9% yield) as a yellow liquid.

[0890] ¹H-NMR (400 MHz, CDCl₃)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, *J* = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H),
10 0.88 (m, 9H). ELSD analysis: Purity 97.90 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.35 (m/z, M+H⁺).

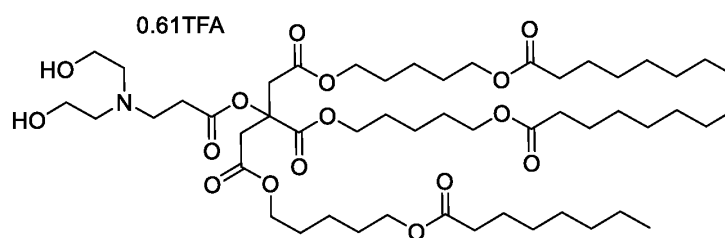
Intermediate [RR-7]:



[0891] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [RR-5] (10 g, 12.1 mmol) in triethylamine (6.10 g, 60.3 mmol) added 3-bromopropanoyl chloride (1.21 mL, 12.1 mmol) was successively at 0 °C and stirred for 16 h, at same 25 °C temperature. TLC shows SM consumed and formed new spot. The reaction mass was diluted with ethyl acetate, filtered through celite and washed with ethyl acetate to get crude product. The crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in
20 Hexanes), to give the desired [RR-7] (2.0 g, yield 18.7%) as a yellow liquid.

[0892] ¹H NMR (400 MHz, CDCl₃): δ 6.42 (d, *J* = 17.2 Hz, 1H), 6.12-6.05 (m, 1H), 5.89 (d, *J* = 10.4 Hz, 1H), 4.17 (t, *J* = 6.4 Hz, 2 H), 4.09-4.03 (m, 10H), 3.34 (d, *J* = 15.2 Hz, 2H), 3.25 (d, *J* = 15.6 Hz, 2H), 2.28 (t, *J* = 7.2 Hz, 6 H), 1.70-1.59 (m, 18H), 1.43-1.35 (m, 6H), 1.28 (s, 24H), 0.87 (t, *J* = 6.8 Hz, 9 H). ELSD analysis: Purity 99.84%, Calculated C₄₈H₈₂O₁₄ = 882.57, Observed = 883.40 (m/z, M+H⁺).

25 **TL1-12D-016 (Compound 105):**



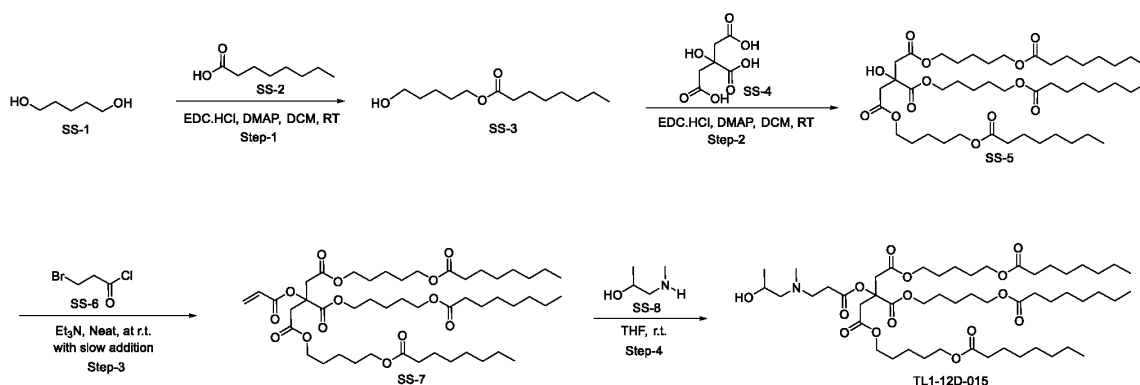
TL1-12D-016

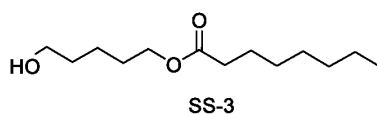
[0893] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate [RR-7] (350 mg, 396 μmol) in tetrahydrofuran (5 mL, 61.4 mmol) was added 2-[(2-hydroxyethyl)amino]ethan-1-ol [RR-8] (38 μL , 396 μmol). Reaction mass was stirred at RT for 48 h. Progress of the reaction was monitored by TLC. Reaction mass was distilled out under reduced pressure and resulting crude material was purified by Prep HPLC. The pure fraction were combined and concentrated under reduced pressure to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-({3-[bis(2-hydroxyethyl)amino]propanoyl}oxy)propane-1,2,3-tricarboxylate [TL1-12D-016] (350 mg, 89.36% Yield) as a yellow color liquid.

[0894] $^1\text{H-NMR}$ (400 MHz, CDCl_3) - δ 4.17 (t, J = 6.8 Hz, 2H), 4.10-4.04 (m, 10H), 4.02-4.00 (m, 4H), 3.60 (t, J = 7.2 Hz, 2H), 3.36-3.34 (m, 4H), 3.24 (d, J = 15.6 Hz, 2H), 3.15 (d, J = 15.6 Hz, 2H), 2.94 (t, J = 7.2 Hz, 2H), 2.28 (t, J = 6.4 Hz, 6H), 1.70-1.58 (m, 18H), 1.44-1.36 (m, 6H), 1.31-1.24 (m, 24H), 0.87 (t, J = 6.8 Hz, 9H). ELSD analysis: Purity 99.73 %, Calculated $\text{C}_{52}\text{H}_{93}\text{NO}_{16}$ = 987.65, Observed = 988.65 (m/z , $\text{M}+\text{H}^+$).

Example 40D. Synthesis of TL1-12D-015 (Compound 103):

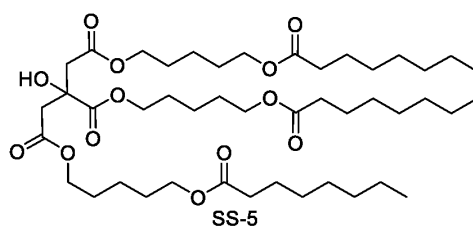
Synthetic Scheme RR



Intermediate [SS-3]:

[0895] To a stirred solution of octanoic acid [SS-2] (100.0 g, 693 mmol) and pentane-1,5-diol [SS-1] (65.0 g, 624 mmol) in dichloromethane (2000 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl] imino)methylidene(ethyl)amine hydrochloride (167.0 g, 874 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (17.1 g, 139 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture, and extract with DCM (3x500 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10% Ethyl acetate in Hexane), to give 5-hydroxypentyl octanoate [SS-3] (60.0 g, yield 37.56%) as a yellow liquid.

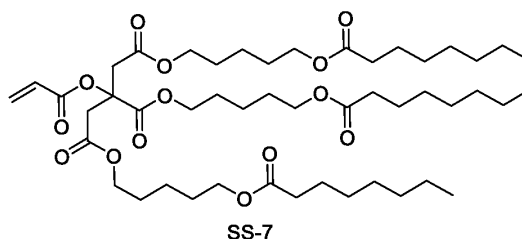
[0896] ¹H-NMR (400 MHz, CDCl₃) δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 5H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

Intermediate [SS-5]:

[0897] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [SS-4] (12.5 g, 65.1 mmol) and 5-hydroxypentyl octanoate [SS-3] (60.0 g, 260 mmol) in dichloromethane (500 mL) was cooled to 0 °C, and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (49.9 g, 260 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (8.02 g, 65.1 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [SS-5] (21.0 g, yield 38.9%) as a yellow liquid.

[0898] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H). ELSD analysis: Purity 97.90 %, Calculated $\text{C}_{45}\text{H}_{80}\text{O}_{13}$ = 828.56, Observed = 829.35 (m/z , $\text{M}+\text{H}^+$).

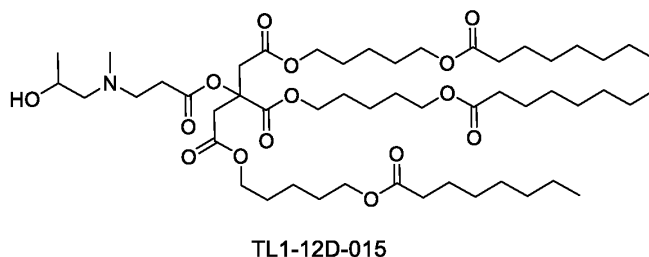
5 **Intermediate [SS-7]:**



[0899] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [SS-5] (10 g, 12.1 mmol) in triethylamine (6.10 g, 60.3 mmol) added 3-bromopropanoyl chloride (1.21 mL, 12.1 mmol) was successively at 0 °C and stirred for 16 h, at 25 °C temperature. TLC showed SM consumed and formation of new spot. The reaction mass was filtered through celite and washed with ethyl acetate to get crude product. The crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired compound [SS-7] (2.0 g, yield 18.7%) as a yellow liquid.

[0900] $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 6.42 (d, J = 17.2 Hz, 1H), 6.12-6.05 (m, 1H), 5.89 (d, J = 10.4 Hz, 1H), 4.17 (t, J = 6.4 Hz, 2 H), 4.09-4.03 (m, 10H), 3.34 (d, J = 15.2 Hz, 2H), 3.25 (d, J = 15.6 Hz, 2H), 2.28 (t, J = 7.2 Hz, 6 H), 1.70-1.59 (m, 18H), 1.43-1.35 (m, 6H), 1.28 (s, 24H), 0.87 (t, J = 6.8 Hz, 9 H). ELSD analysis: Purity 99.84%, Calculated $\text{C}_{48}\text{H}_{82}\text{O}_{14}$ = 882.57, Observed = 883.40 (m/z , $\text{M}+\text{H}^+$).

TL1-12D-015 (Compound 103):



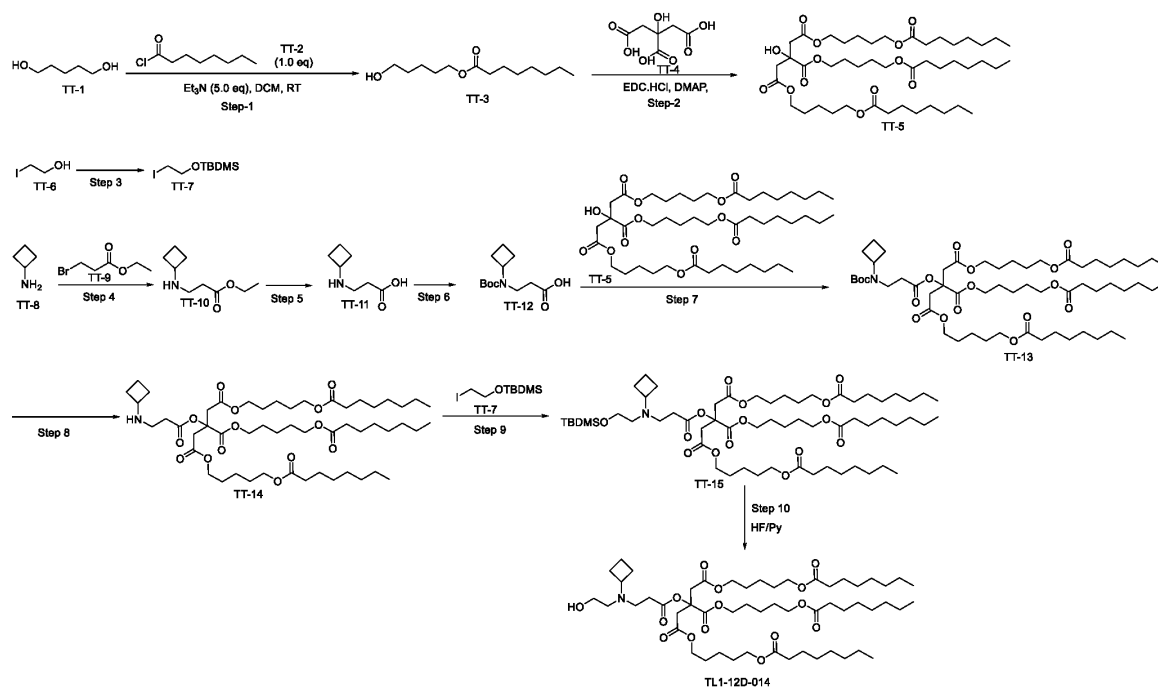
[0901] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate [SS-7] (712 mg, 806 μmol) in tetrahydrofuran (20 mL, 246 mmol) was added 1-(methylamino)propan-2-ol [SS-8] (71.9 mg, 806 μmol). Reaction mass was stirred at RT for 48 h.

Progress of the reaction was monitored by TLC. Reaction mass was distilled out under reduced pressure and resulting crude material was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexanes), to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-({3-[(2-hydroxypropyl)(methyl)amino] propanoyl}oxy)propane-1,2,3-tricarboxylate [TL1-12D-015] (0.3 g, Yield 38.27%) as a yellow liquid.

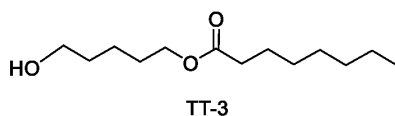
[0902] ¹H-NMR (400 MHz, CDCl₃)- δ 4.16 (t, *J* = 6.4 Hz, 2H), 4.09-4.04 (m, 10H), 3.79 (bs, 1H), 3.31-3.19 (m, 4H), 2.89-2.79 (m, 1H), 2.63-2.58 (m, 1H), 2.50-2.44 (m, 2H), 2.30-2.26 (m, 10H), 1.72-1.57 (m, 20H), 1.44-1.38 (m, 6H), 1.29-1.27 (m, 24H), 1.10 (d, *J* = 6.0 Hz, 3H), 0.87 (t, *J* = 6.8 Hz, 9H). ELSD analysis: Purity 99.75 %, Calculated C₅₂H₉₃NO₁₅ = 971.65, Observed = 972.60 (*m/z*, M+H⁺).

Example 40E. Synthesis of TL1-12D-014 (Compound 104):

Synthetic Scheme SS



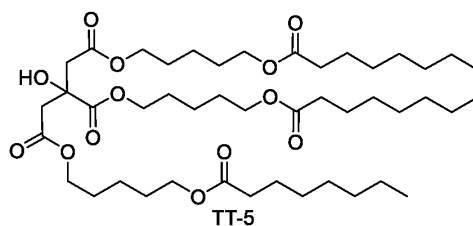
Intermediate [TT-3]:



[0903] To a stirred solution of pentane-1,5-diol (64 g, 615 mmol) in dichloromethane (0.5 L, 7.81 mol) was added octanoyl chloride (50 g, 307 mmol) and triethylamine (156 g, 1.54 mol). The reaction mixture stirred at r.t. for 24 h. The progress of reaction was monitored by TLC (SM was consumed). Reaction mixture was quenched by cold saturated NaHCO_3 upto pH 7 and extracted with DCM (2x500 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-20 % ethyl acetate in hexane), to afford 5-hydroxypentyl octanoate (45 g, yield 63.5 %) as a pale-yellow liquid.

[0904] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.07 (t, $J = 6.4$ Hz, 2H), 3.65 (t, $J = 6.4$ Hz, 2H), 2.28 (m, $J = 7.6$ Hz, 2H), 1.69-1.56 (m, 4H), 1.47-1.41 (m, 2H), 1.39-1.2 (m, 9H), 0.87 (t, $J = 7.2$ Hz, 3H).

Intermediate [TT-5]:

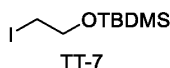


[0905] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [TT-4] (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate [TT-3] (6 g, 26 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [TT-5] (2.1 g, yield 38.9%) as a pale yellow liquid.

[0906] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, $J = 15.6, 19.2$ Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H),

0.89-0.85 (m, 9H). ELSD analysis: Purity 97.90 %, Calculated $C_{45}H_{80}O_{13}$ = 828.56, Observed = 829.35 (m/z, $M+H^+$).

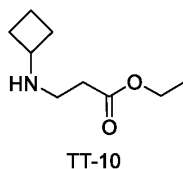
Intermediate [TT-7]:



- 5 **[0907]** To a stirred solution of 2-iodoethan-1-ol **[TT-6]** (5 g, 0.0290 mol) in dichloromethane (100 mL), 1*H*-imidazole (1.97 g, 0.0290 mol) was added at room temperature. The resulting reaction mixture was cooled to 0 °C and tert-butyl(chloro)dimethylsilane (8.76 g, 0.0580 mol) was added portion wise at same temperature. The reaction mixture was stirred for 16 h at room temperature. Progress of reaction was monitored by ELSD/TLC. Water (50 mL) was added to the
- 10 reaction mixture and extract with DCM (3x100 mL). The resulting organic layer was dried over anhydrous sodium sulphate and concentrated under reduce pressure to obtain crude product which was purified by flash column chromatography (0-10 % ethyl acetate in n-hexane), to give pure product **[TT-7]** (5.0 g, yield 60.1 %) as a colorless liquid.

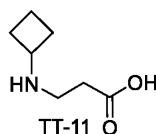
- [0908]** 1H -NMR (400 MHz, $CDCl_3$): δ 3.83 (t, J = 5.6 Hz, 2H), 3.19 (t, J = 6.8 Hz, 2H), 0.90 (s, 9H),
- 15 0.10 (s, 6H).

Intermediate [TT-10]:



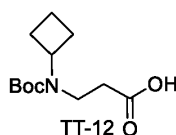
- [0909]** To a stirred solution of cyclobutanamine **[TT-8]** (5 g, 70.3 mmol) in acetonitrile (250 mL, 4.79 mol), dipotassium carbonate (29.1 g, 211 mmol), and ethyl 3-bromopropanoate **[TT-9]** (12.7
- 20 g, 70.3 mmol) was added at room temperature, and reaction was allow to stir at 60 °C for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, reaction mass was cooled, filter by sintered glass funnel. The filtrate was concentrated under reduced pressure and the crude was purified by flash column chromatography (SiO_2 : 0-10% Methanol in Dichloromethane), to give ethyl 3-(cyclobutylamino)propanoate **[TT-10]** (4.5 g, 37.38% Yield) as
- 25 pale yellow liquid.

- [0910]** 1H -NMR (400 MHz, $CDCl_3$): δ 4.10 (q, J = 7.2 Hz, 2H), 3.26-3.19 (m, 1H), 2.77(t, J = 6.8 Hz, 2H), 2.46 (t, J = 6.8 Hz, 2H), 2.21-2.14 (m, 3H), 1.75-1.63 (m, 3H), 1.24-1.20 (m, 3H).

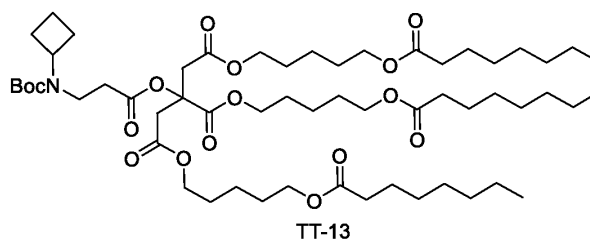
Intermediate [TT-11]:

- [0911] To a stirred suspension of ethyl 3-(cyclobutylamino)propanoate [TT-10] (4 g, 23.4 mmol) in ethanol (40 mL, 685 mmol), lithium(1+) hydrate hydroxide (1.08 g, 25.7 mmol) was added at room temperature, The reaction mixture was stirred reaction mixture for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, reaction mixture was concentrated under reduced pressure. The crude material was acidified by 1 N HCl up to pH 5 and extracted with ethyl acetate. The resulting organic layer was dried over anhydrous sodium sulphate and concentrated under reduce pressure to obtain 3-(cyclobutylamino)propanoic acid [TT-11] (3.34 g, yield 99.86%), compound was used for next step.

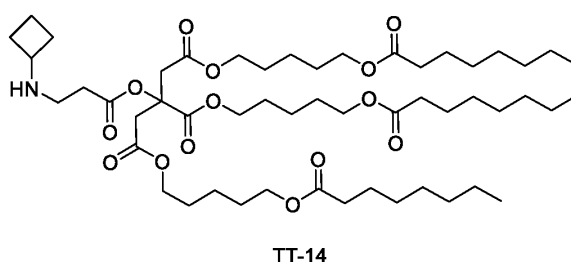
[0912] *ELSD analysis:* Purity 77 %, Calculated $C_7H_{13}NO_2 = 143.09$, Observed = 144.25 (m/z, $M+H^+$).

Intermediate [TT-12]:

- [0913] To a stirred solution of 3-(cyclobutylamino)propanoic acid [TT-11] (3.2 g, 22.32 mmol), and potassium hydroxide (2.5 g, 44.72 mmol) in methanol (20 mL, 494 mmol) and water (20 mL, 1110 mmol) was added di-tert-butyl dicarbonate (7.32 g, 33.52 mmol) at room temperature and stirred for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, mixture was concentrated under reduced pressure. The crude material was acidified by 10 % $NaHSO_4$ up to pH 2-3 and extracted with ethyl acetate (3x200 mL). The resulting organic layer was dried over anhydrous sodium sulphate concentrated under reduced pressure to obtained crude product which was purified by flash column chromatography (0-100 % ethyl acetate in n-hexane), to obtain 3-[(tert-butoxy)carbonyl](cyclobutyl)amino)propanoic acid [TT-12] (4.0 g, yield 73.56 %) as a colorless liquid.
- [0914] 1H -NMR (400 MHz, $CDCl_3$): δ 4.29 (bs, 1H), 3.51-3.48 (m, 2H), 2.57 (t, $J = 7.2$ Hz, 2H), 2.17-2.10 (m, 2H), 2.09-1.99 (m, 2H), 1.66-1.54 (m, 2H), 1.45 (s, 9H) ppm.

Intermediate [TT-13]:

- [0915]** To a stirred solution of 3-[[[tert-butoxy]carbonyl](cyclobutyl)amino]propanoic acid [TT-12] (2.11 g, 8.68 mmol) and 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [TT-5] (1.8 g, 2.17 mmol) in dichloromethane (45 mL, 703 mmol) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (1.66 g, 8.68 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (1.07 g, 8.68 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture and extracted with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes) to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[[3-[[[tert-butoxy]carbonyl](cyclobutyl)amino]propanoyl]oxy]propane-1,2,3-tricarboxylate [TT-14] (1.9 g, yield 83%) as pale yellow liquid.
- [0916]** *ELSD analysis:* Purity 97.56 %, Calculated C₅₇H₉₉NO₁₆ = 1053.70, Observed = 1076.65 (m/z, M+Na⁺).

Intermediate [TT-14]:

- [0917]** To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[[3-[[[tert-butoxy]carbonyl](cyclobutyl)amino]propanoyl]oxy]propane-1,2,3-tricarboxylate [TT-13] (1.9 g, 1.8 mmol) in dichloromethane (20 mL, 312 mmol), trifluoroacetic acid (1.38 mL, 10 eq., 18 mmol) was added at 0 °C. The resulting reaction mixture was stirred for 6 h at room temperature. The progress of

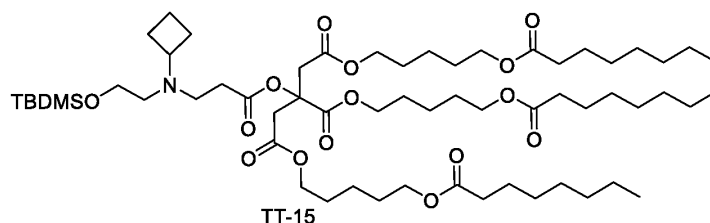
reaction mass was monitored by ELSD/TLC (SM was consumed). After completion the reaction, mixture was concentrated under reduced pressure. The crude material was quenched by NaHCO_3 upto pH 8 and extracted with ethyl acetate (3x50 mL). The resulting organic layer was dried over anhydrous sodium sulphate, concentrated under reduced pressure to obtain 1,2,3-tris[5-

5 (octanoyloxy)pentyl] 2-[[3-(cyclobutylamino)propanoyl]oxy]propane-1,2,3-tricarboxylate [TT-14] (850 mg, yield 49.43 %) as a yellow liquid, which was used in next step as such.

[0918] *ELSD analysis:* Purity 99.45 %, Calculated $\text{C}_{52}\text{H}_{91}\text{NO}_{14}$ = 953.64, Observed = 954.60 (m/z, $\text{M}+\text{H}^+$).

Intermediate [TT-15]:

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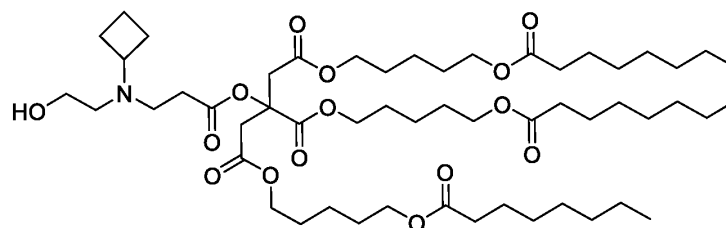
[0919] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-(cyclobutylamino)propanoyl] oxy]propane-1,2,3-tricarboxylate [TT-14] (750 mg, 786 μmol) in acetonitrile (15 mL), dipotassium carbonate (543 mg, 3.93 mmol), and tert-butyl(2-

15 iodoethoxy)dimethylsilane [TT-7] (270 mg, 943 μmol) was added at room temperature. The reaction mixture was stirred at 60 °C for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, reaction mass was cooled, filter by sintered glass funnel. The filtrate was concentrated under reduced pressure and the crude was purified by flash column chromatography (SiO_2 : 0-10% Methanol in Dichloromethane), to give 1,2,3-tris[5-

20 (octanoyloxy)pentyl] 2-[[3-([2-[(tert-butyldimethylsilyl)oxy]ethyl](cyclobutyl)amino)propanoyl]oxy]propane-1,2,3-tricarboxylate [TT-15] (290 g, 33.16% Yield) as colorless liquid.

[0920] *ELSD analysis:* Purity 76.81 %, Calculated $\text{C}_{60}\text{H}_{109}\text{NO}_{15}\text{Si}$ = 1111.76, Observed = 1112.75 (m/z, $\text{M}+\text{H}^+$).

[TL1-12D-014] (Compound 104):



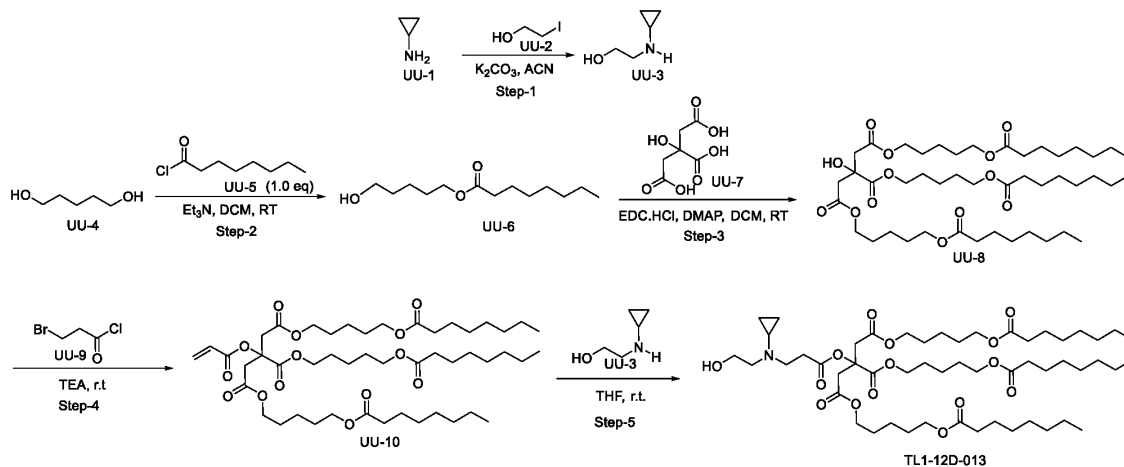
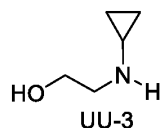
TL1-12D-014

[0921] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[[3-({2-[(tert-butyl)dimethylsilyl]oxy}ethyl) (cyclobutyl)amino)propanoyl]oxy]propane-1,2,3-tricarboxylate [TT-15] (330 mg, 297 μ mol) in tetrahydrofuran (4 mL, 49.1 mmol) was added pyridine hydrofluoride (0.2 mL, 1.48 mmol) at 0°C. The resulting reaction mixture was stirred for 2 h at room temperature. Progress of reaction mass was monitored by ELSD/TLC (SM was consumed). The resulting reaction mixture was quenched with cold aqueous sodium bicarbonate solution (20 mL) and extract with ethyl acetate (3x25 mL). The resulting organic layer was dried over Na₂SO₄ and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-10 % methanol in dichloromethane), to obtain desired produced [TL1-12D-014] (86 mg, yield 29.04 %) as a yellow color liquid.

[0922] ¹H-NMR (400 MHz, CDCl₃)- δ 4.16 (t, *J* = 6.8 Hz, 2H), 4.09-4.04 (m, 10H), 3.54-3.52 (m, 2H), 3.30 (d, *J* = 15.6, 2H), 3.22 (d, *J* = 15.6, 2H), 3.14-3.06 (m, 1H), 2.77-2.74 (m, 2H), 2.52-2.49 (m, 2H), 2.45-2.42 (m, 2H), 2.28 (t, *J* = 7.6 Hz, 6H), 2.05-1.98 (m, 2H), 1.91-1.82 (m, 2H), 1.71 (m, 20H), 1.44-1.37 (m, 6H), 1.32-1.24 (m, 24H), 0.87 (t, *J* = 6.4 Hz, 9H) ppm. ELSD analysis: Purity 98.79 %, Calculated C₅₄H₉₅NO₁₅ = 997.67, Observed = 998.55 (*m/z*, M+H⁺).

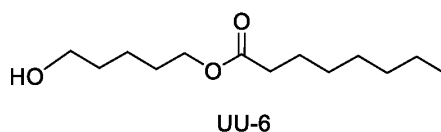
Example 40F. Synthesis of TL1-12D-013 (Compound 108):

Synthetic Protocol TT

**Intermediate [UU-3]:**

- [0923] To a stirred solution of cyclopropanamine [UU-1] (1 g, 17.5 mmol), and 2-iodoethan-1-ol [UU-2] (3.01 g, 17.5 mmol) in acetonitrile (20 mL, 383 mmol) at 60 °C for 16 h. The progress of reaction was monitored by TLC. After completion the reaction, reaction mass was filtered by sintered funnel. The filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (0-20 % MeOH/Dichloromethane), to afford 2-(cyclopropylamino)ethan-1-ol [UU-3] (1.6 g, yield 90 %) as a pale yellow liquid.

- [0924] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 4.08 (t, J = 5.2 Hz, 2H), 3.35 (br, 2H), 2.75 (br, 1H), 0.99-0.95 (m, 4H).

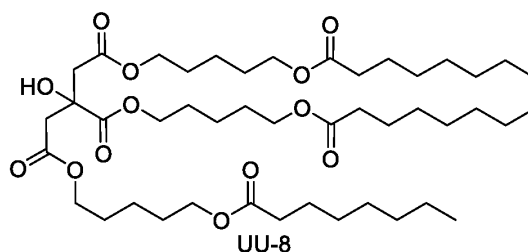
Intermediate [UU-6]:

- [0925] To a stirred solution of pentane-1,5-diol [UU-4] (64 g, 615 mmol) in dichloromethane (0.5 L, 7.81 mol), was added octanoyl chloride [UU-5] (50 g, 307 mmol) and triethylamine (156 g, 1.54 mol). The reaction mixture stirred at r.t. for 24 h. The progress of reaction was monitored by TLC (SM was consumed). Reaction mixture was quenched by cold saturated NaHCO_3 up to pH7 and extracted with DCM (2x500 mL). The resulting organic layer was dried over Na_2SO_4 , and

concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-20 % ethyl acetate in hexane), to afford 5-hydroxypentyl octanoate [UU-6] (45 g, 63.5 % Yield) as a pale-yellow liquid.

[0926] ¹H-NMR (400 MHz, CDCl₃)- δ 4.07 (t, *J* = 6.4 Hz, 2H), 3.65 (t, *J* = 6.4 Hz, 2H), 2.28 (m, *J* = 7.6 Hz, 2H), 1.69-1.56 (m, 4H), 1.47-1.41 (m, 2H), 1.39-1.2 (m, 9H), 0.87 (t, *J* = 7.2 Hz, 3H).

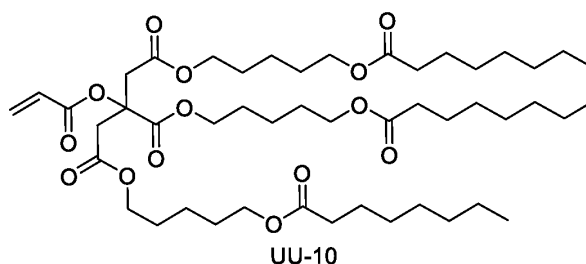
Intermediate [UU-8]:



[0927] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [UU-7] (1.25 g, 6.51 mmol) and 5-hydroxypentyl octanoate [UU-6] (6 g, 26 mmol) in dichloromethane (50 mL, 781 mmol) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (4.99 g, 26 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (802 mg, 6.51 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [UU-8] (2.1 g, 38.9% yield) as pale yellow liquid.

[0928] ¹H-NMR (400 MHz, CDCl₃)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, *J* = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.89-0.85 (m, 9H). *ELSD analysis*: Purity 97.90 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.35 (m/z, M+H⁺).

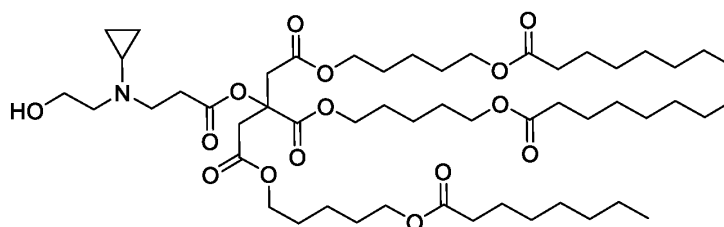
Intermediate [UU-10]:



[0929] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [UU-8] (5 g, 6.05 mmol) in triethylamine (3.05 g, 18.15 mmol) was added 3-bromopropanoyl chloride [UU-9] (605 μ L, 6.05 mmol) was successively at 0 °C and stirred for 16h at same 25 °C temperature, TLC shows SM consumed and formed new spot. The reaction mass was diluted, filtered through celite and washed with ethyl acetate to get crude product. The crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give tris(5-(octanoyloxy)pentyl) 2-(acryloyloxy)propane-1,2,3-tricarboxylate [UU-10] (1.0 g, yield 18.7%) as yellow liquid.

[0930] ¹H NMR (400 MHz, CDCl₃): δ 6.44-6.40 (d, J = 17.2 Hz, 1H), 6.12-6.05 (dd, J = 10.4 Hz, J = 6.8 Hz, 1H), 5.98-5.88 (d, J = 10.4 Hz, 1H), 4.17 (t, J = 6.4 Hz, 2 H), 4.09-4.03 (m, 10 H), 3.36-3.23 (dd, J = 15.2 Hz, J = 15.6 Hz, 4H), 2.30-2.26 (t, J = 7.2 Hz, 6 H), 1.70-1.55 (m, 16H), 1.43-1.35 (m, 6H), 1.28 (s, 26H), 0.87 (t, J = 6.8 Hz, 9 H).

TL1-12D-013 (Compound 108):



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[0931] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate [UU-10] (350 mg, 396 μ mol) in tetrahydrofuran (2 mL, 24.6 mmol), was added 2-(cyclopropylamino)ethan-1-ol [UU-3] (60.1 mg, 594 μ mol). Reaction mass was stirred at RT for 48 h. Progress of the reaction was monitored by TLC. After completion of reaction, concentrated the reaction mixture under reduce pressure, and the crude was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexanes), to give 1,2,3-tris[5-

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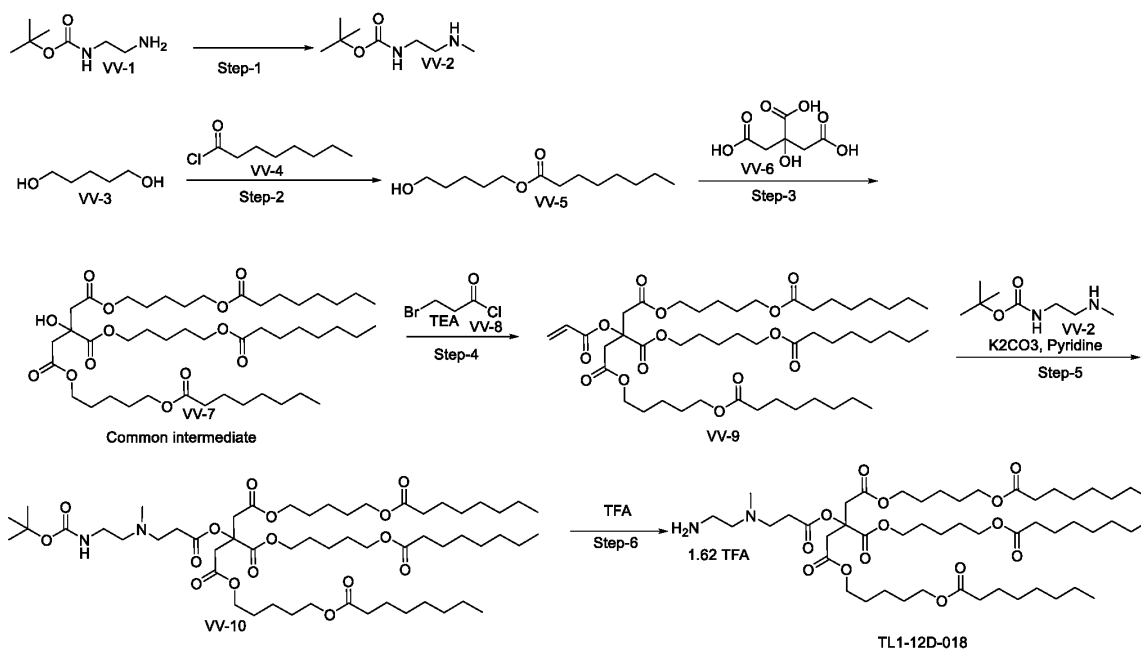
(octanoyloxy)pentyl] 2-({3-[cyclopropyl(2-hydroxyethyl)amino]propanoyl}oxy)propane-1,2,3-tricarboxylate [TL1-12D-013] (0.11g, 28.4 % yield) as a colorless liquid.

[0932] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.18 (t, J = 6.4 Hz, 2H), 4.09-4.04 (m, 10H), 3.58 (s, 2H), 3.30 (d, J = 15.6 Hz, 2H), 3.20 (d, J = 15.6 Hz, 2H), 2.89 (t, J = 6.8 Hz, 2H), 2.73 (t, J = 5.2 Hz, 2H), 2.56 (t, J = 6.8 Hz, 2H), 2.28 (t, J = 7.6 Hz, 6H), 1.72-1.59 (m, 20H), 1.45-1.36 (m, 6H), 1.29-1.27 (m, 23H), 0.87 (t, J = 6.8 Hz, 9H), 0.51-0.42 (m, 4H). ELSD analysis: Purity 99.71 %, Calculated $\text{C}_{53}\text{H}_{93}\text{NO}_{15}$ = 983.65, Observed = 984.50 (m/z , $\text{M}+\text{H}^+$).

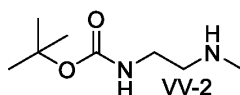
Example 40G. Synthesis of TL1-12D-018 (Compound 109):

Synthetic Protocol UU

10



Intermediate [VV-2]:



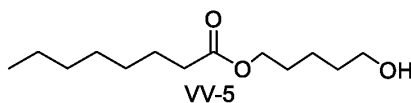
[0933] To a stirrer solution of tert-butyl N-(2-aminoethyl)carbamate [VV-1] (2 g, 12.5 mmol) in tetrahydrofuran (0.4 L, 4.91 mol) added triethylamine (1.75 mL, 12.5 mmol) and iodomethane (1.77 g, 12.5 mmol) dropwise. reaction mass was stirrer for 48 h at RT. Reaction progress was

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monitored by TLC. As starting material consumed, reaction mass was distilling out under reduced pressure. The resulting crude was purified with column chromatography using 0-5% methanol in DCM, to give tert-butyl N-[2-(methylamino)ethyl]carbamate **[VV-2]** (0.8 g, 30 % Yield) as a brown color semi solid.

- 5 **[0934]** ^1H NMR (400 MHz, CDCl_3): δ 3.70-3.56 (m, 2H), 2.76 (s, 3H), 1.45 (s, 9H), 1.28-1.23 (m, 2H). ELSD Purity - 81.71%

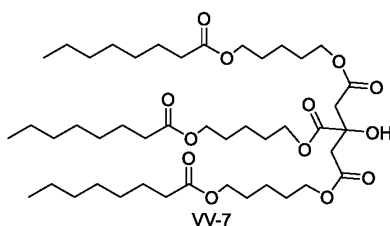
Intermediate [VV-5]:



- [0935]** To a stirred solution of pentane-1,5-diol **[VV-3]** (50 g, 4.9 eq., 480 mmol) in dichloromethane (205 mL, 3.2 mol), was added triethylamine (69.1 mL, 5 eq., 492 mmol) and N,N-dimethylpyridin-4-amine (6.01 g, 0.5 eq., 49.2 mmol) at RT. Reaction mass cooled to 0-5°C, then dropwise added octanoyl chloride **[VV-4]** (16 g, 98.4 mmol). Reaction mixture allowed to stir at RT for 16 h. Progress of reaction was monitored by TLC. Reaction mixture was diluted with water (2.0 Lit) and extracted with ethyl acetate (200 ml). Organic layer was washed with brine solution (500.0 mL), dried over anhydrous sodium sulphate, filtered and concentrate under reduced pressure. Crude was purified over silica gel with column chromatography using 5-10% Ethyl acetate in hexanes to give 5-hydroxypentyl octanoate **[VV-5]** (16 g, 70.6 % yield) as light yellow colour liquid.
- 10
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- [0936]** ^1H NMR (400 MHz, CDCl_3): δ 4.07 (t, J = 6.8 Hz, 2H), 3.66 (s, 2H), 2.30 (t, J = 7.6 Hz, 2H), 1.69-1.58 (m, 8H), 1.44 (m, 2H), 1.29 (s, 10H), 0.87 (t, J = 7.2 Hz, 3H).
- 20

Intermediate [VV-7]:



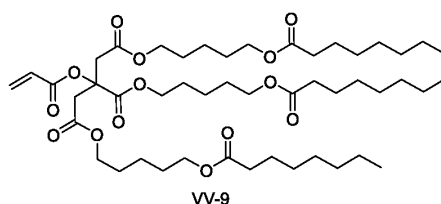
- [0937]** To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid **[VV-6]** (2.5 g, 13 mmol) in dichloromethane (50 mL, 781 mmol), was added N,N-dimethylpyridin-4-amine (1.59 g, 13 mmol) and ([{3-(dimethylamino)propyl}imino)methylidene)(ethyl)amine hydrochloride (9.98 g,
- 25

4 eq., 52 mmol) and stirred for 10 min at RT, then added 5-hydroxypentyl octanoate [VV-5] (10.5 g, 3.5 eq., 45.5 mmol). Reaction mixture was stirred for 16 h at RT under inert atmosphere.

Reaction progress was monitored by TLC, after consumption of starting material reaction mass diluted with water (100.0 mL) and extracted with dichloromethane (2x 100.0 mL). Organic layer was separated, dried over sodium sulphate, distil out under reduced pressure. Crude purified with flash chromatography using 10-15% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [VV-7] (3.5 g, 4.22 mmol) as a light yellow color liquid.

[0938] ^1H NMR (400 MHz, CDCl_3): δ 4.22 (t, J = 6.4 Hz, 2H), 4.09 (m, 10H), 2.89-2.77 (dd, J = 15.6 Hz, 4H), 2.28 (t, J = 7.6 Hz, 6H), 1.64 (m, 18H), 1.43 (m, 6H), 1.29 (m, 24H), 0.87 (t, J = 7.2 Hz, 9H).

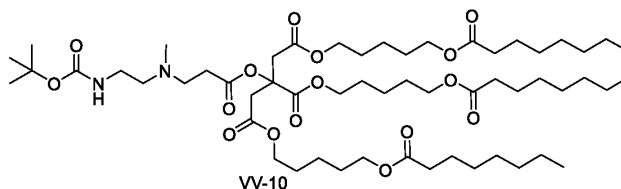
Intermediate [VV-9]:



[0939] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [VV-7] (1 g, 1.21 mmol) in triethylamine (610 mg, 5 eq., 6.03 mmol), was added 3-bromopropanoyl chloride [VV-8] (121 μL , 1.21 mmol) 0 °C and stirred for 16 h at 25 °C temperature. Progress of TLC shows SM consumed and formed new spot. The reaction mass was filtered through celite and washed with ethyl acetate to get crude product. crude was purified by using flash column under 15-25% ethyl acetate in hexane to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enyloxy)propane-1,2,3-tricarboxylate [VV-9] (0.20 g, 18.78 % yield).

[0940] ELSD: Purity 99.84 %, Calculated $\text{C}_{48}\text{H}_{82}\text{O}_{14}$ = 882.57, Observed = 883.40 (m/z , $\text{M}+\text{H}^+$).

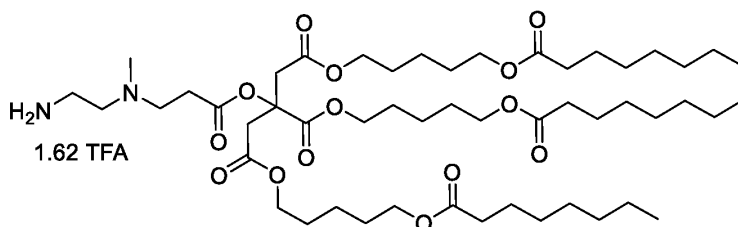
Intermediate [VV-10]:



[0941] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate (850 mg, 962 μmol) in tetrahydrofuran (20.6 mL, 254 mmol). Added tert-butyl N-[2-(methylamino) ethyl] carbamate (335 mg, 2 eq., 1.92 mmol). Reaction mass was stirred at RT. Progress of the reaction was monitored by TLC. Reaction mass was distilling out under reduced pressure, get crude. The resulting crude was purified with column chromatography using 5% methanol in Dichloromethane, to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-({3-[(2-[(tert-butoxy)carbonyl]amino)ethyl]amino]propanoyl}oxy)propane-1,2,3-tricarboxylate (200 mg, 189 μmol) as a yellow color liquid.

[0942] ELSD: Purity 99.15 %, Calculated $\text{C}_{56}\text{H}_{100}\text{N}_2\text{O}_{16} = 1056.71$, Observed = 1057.71 (m/z , $\text{M}+\text{H}^+$).

TL1-12D-018 (Compound 109):



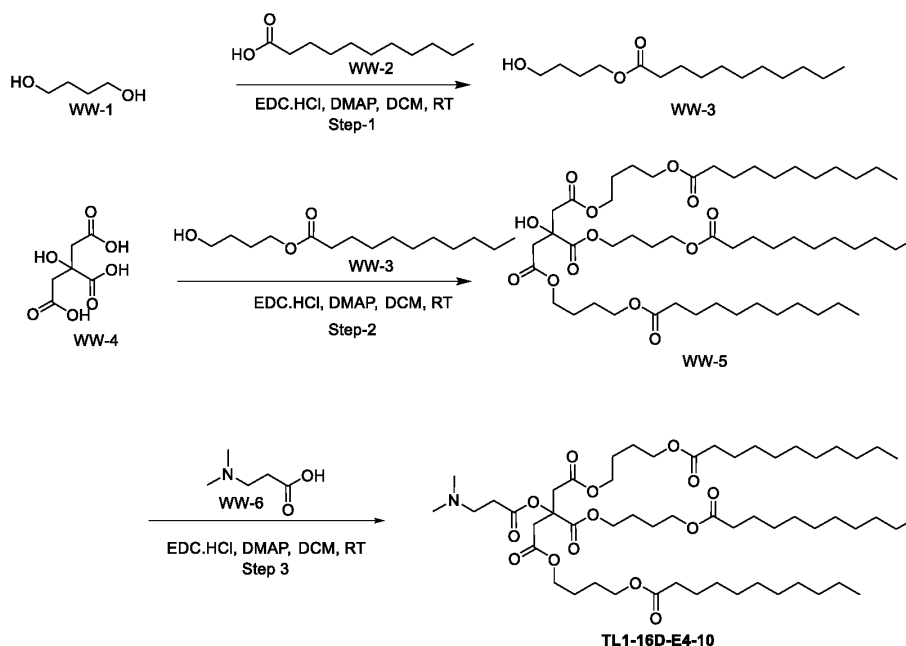
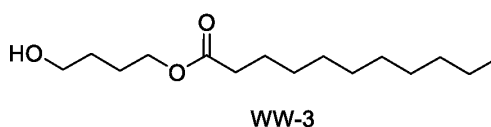
TL1-12D-018

[0943] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-({3-[(2-[(tert-butoxy)carbonyl]amino)ethyl](methyl)amino]propanoyl}oxy)propane-1,2,3-tricarboxylate [VV-10] (450 mg, 426 μmol) in dichloromethane (10 mL, 156 mmol), was added trifloroacetic acid (97 mg, 2 eq., 851 μmol) at 0 °C. Reaction mass allowed to stir at RT for 2 h. Progress of the reaction was monitored by TLC and ELSD. SM was consumed. Reaction mass was lyophilized to obtain 1,2,3-tris[5-(octanoyloxy)pentyl] 2-({3-[(2-aminoethyl)(methyl)amino]propanoyl}oxy)propane-1,2,3-tricarboxylate [TL1-12D-018] (250 mg, 261 μmol) as brown liquid.

[0944] ^1H NMR (400 MHz, CDCl_3): δ 4.18 (t, $J = 6.4$ Hz, 2H), 4.09-4.04 (q, $J = 9.2$ Hz, 10H), 3.62-3.50 (d, $J = 8.0$ Hz, 4H), 3.45 (t, $J = 6.4$ Hz, 2H), 3.25-3.14 (dd, $J = 15.6$ Hz, $J = 15.6$ Hz, 4H), 2.88 (m, 4H), 2.30 (t, $J = 7.6$ Hz, 6H), 1.73-1.55 (m, 18 H), 1.45-1.36 (m, 6H), 1.32-1.25 (brs, 24H), 0.87 (t, $J = 6.4$ Hz, 9H). ELSD Purity 97.62 %, Calculated $\text{C}_{51}\text{H}_{92}\text{N}_2\text{O}_{14} \cdot \text{C}_2\text{HF}_3\text{O}_2 = 957.65$, Observed = 957.50 (m/z , $\text{M}+\text{H}^+$).

Example 40H. Synthesis of JC-TL1-16D-E4-10 (Compound 129)

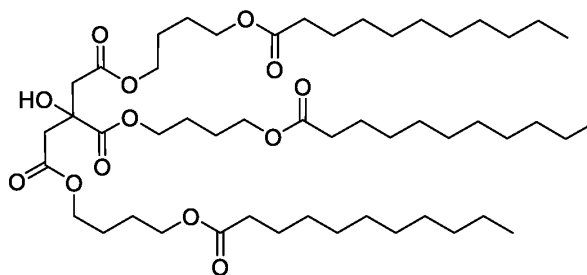
Synthetic Protocol VV

**Intermediate [WW-3]:**

- 5 **[0945]** To a stirred solution of butane-1,4-diol [WW-1] (10 g, 0.111 mol) and undecanoic acid [WW-2] (20.6 g, 0.111 mol) in dichloromethane (200 mL) was cooled to 0 °C, EDC.HCl (25.5 g, 0.133 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (1.35 g, 0.011 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (200 mL) was added to the reaction mixture and extract with DCM (3x300 mL).
- 10 The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure to obtained crude product which was purified by flash column chromatography (0-20 % ethyl acetate in n-hexane) to obtained pure product 4-hydroxybutyl undecanoate [WW-3] (6.0 g, 21 % Yield) as a pale-yellow liquid.

- [0946]** ¹H NMR (400 MHz, CDCl₃): δ 4.10 (t, *J* = 6.4 Hz, 2H), 3.67 (br, 2H), 2.30-2.26 (m, 2H), 1.75-1.68 (m, 2H), 1.66-1.57 (m, 4H), 1.44-1.25 (m, 14H), 0.88 (t, *J* = 7.2 Hz, 3H).
- 15

Intermediate [WW-5]:

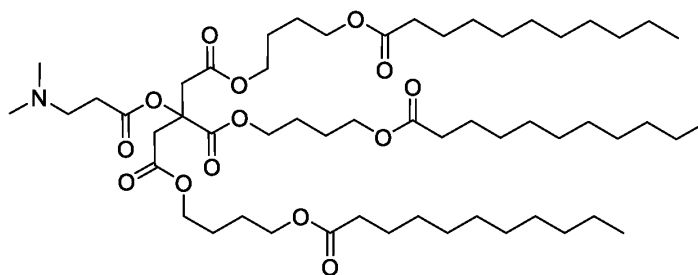


WW-5

- [0947] To a stirred solution of 4-hydroxybutyl undecanoate [WW-3] (5.37 g, 0.0208 mol) and Citric acid [WW-4] (1.0 g, 0.0052 mol) in dichloromethane (150 mL) was cooled to 0 °C, EDC.HCl (4.0 g, 0.0208 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.635 g, 0.0052 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water (100 mL) was added to the reaction mixture and extract with DCM (3x200 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure to obtained crude product which was purified by flash column chromatography (0-30 % ethyl acetate in n-hexane) to obtained pure product tris(4-(undecanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate [WW-5] (1.77 g, 37 % Yield) as a yellow liquid.

[0948] *ELSD analysis:* Purity 96.78 %, Calculated C₅₁H₉₂O₁₃ = 912.65, Observed = 935.35 (m/z, M+Na⁺).

JC-TL1-16D-E4-10 (Compound 129):



JC-TL1-16D-E4-10

- [0949] To a stirred solution of tris(4-(undecanoyloxy)butyl) 2-hydroxypropane-1,2,3-tricarboxylate [WW-5] (0.91 g, 0.0010 mol) and 3-(dimethylamino)propanoic acid [WW-6] (0.466 g, 0.004 mol) in dichloromethane (15 mL) was cooled to 0 °C, EDC.HCl (0.765 g, 0.004 mol), was added followed by 4-(dimethylamino)pyridin-1-ium (0.122 g, 0.0010 mol) and stirred for 48 h at room temperature. The progress of reaction was monitored by TLC (SM was consumed). Water

(50 mL) was added to the reaction mixture and extract with DCM (3x25 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated under reduce pressure to obtained crude product which was purified by flash column chromatography using (0-100 % ethyl acetate in n-hexane) to obtained pure product tris(4-(undecanoyloxy)butyl) 2-((3-

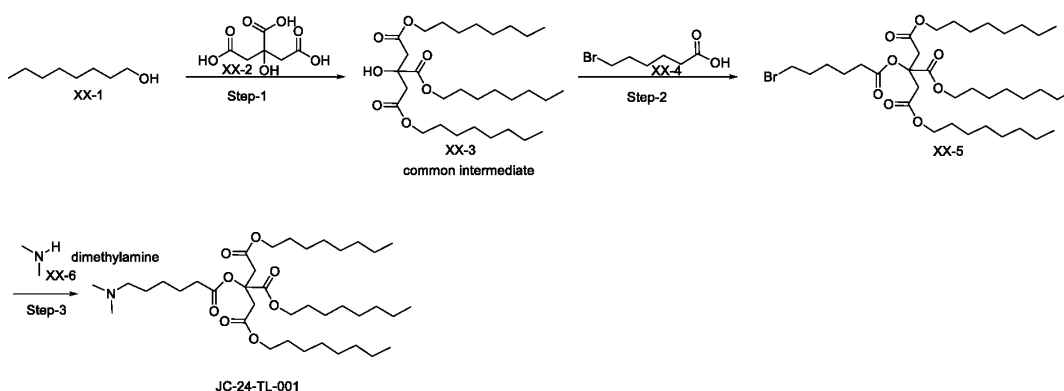
- 5 (dimethylamino)propanoyl)oxy)propane-1,2,3-tricarboxylate [JC-TL1-12D-E4-10] (0.385 g, 38.5 %, Yield) as a brown liquid.

[0950] Results: ¹H NMR (400 MHz, CDCl₃): δ 4.18 (t, *J*= 6.0 Hz, 2H), 4.10-4.08 (m, 10H), 3.30 (d, *J*= 15.6 Hz, 2H), 3.23 (d, *J*= 15.6 Hz, 2H), 2.59-2.54 (m, 2H), 2.51-2.49 (m, 2H), 2.28 (t, *J*= 7.6 Hz, 6H), 2.21 (s, 6H), 1.73-1.67 (m, 12H), 1.62-1.57 (m, 8H), 1.32-1.25 (m, 40H), 0.89-0.85 (m, 9H).

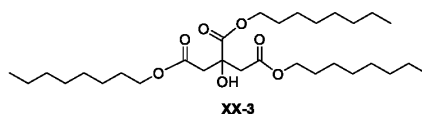
- 10 ELSD analysis: Purity 99.23 %, Calculated C₅₆H₁₀₁NO₁₄ = 1011.72, Observed = 1012.35 (*m/z*, M+H⁺).

Example 40I. Synthesis of JC-24-TL-001 (Compound 118)

Synthetic Protocol WW



Intermediate [XX-3]:



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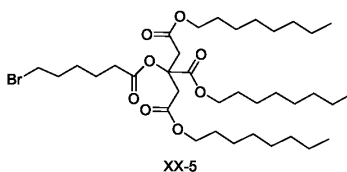
[0951] To a solution of citric acid [XX-2] (25.0 g, 0.130 mol) and octan-1-ol [XX-1] (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol,) was added, and the resulting mixture was stirred at 100 °C for 24 h. The reaction progress was monitored by TLC (5% Ethyl acetate/hexanes). The reaction mixture was cooled to RT, diluted with water (500 mL) and

20 extracted with ethyl acetate (500 mL x 3). The organic layer dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude was purified by column

chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [XX-3] (40.0 g, Yield- 58.18 %) as a colourless liquid.

[0952] ^1H NMR (400 MHz, CDCl_3): δ 5.61 (s, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.4 Hz, 4H), 2.87 (d, J = 15.2 Hz, 2H), 2.72 (d, J = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 97.38 %, Calculated $\text{C}_{30}\text{H}_{56}\text{O}_7$ = 528.77, Observed = 529.2 (m/z , $\text{M}+\text{H}^+$).

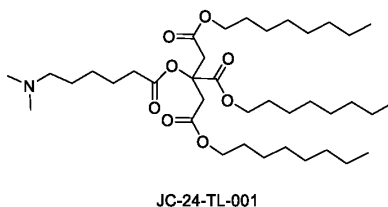
Intermediate [XX-5]:



[0953] To a stirred solution of 6-bromohexanoic acid [XX-4] (4.0 g, 4 eq., 20.5 mmol) in dichloromethane (50 mL), N,N -dimethyl-4-pyridylamine (2.5 g, 20.5 mmol, 4 eq.,) and EDC.HCl (3.93 g, 20.5 mmol, 4 eq.,) were successively added at room temperature and allowed to stir. After 10 minutes trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [XX-3] (2.64 g, 5.13 mmol) was added at room temperature and allowed to stir for 48h. Reaction progress was monitored by TLC/ELSD. SM was consumed. The resulting reaction mixture was quenched with water (100 mL) and extracted with dichloromethane (2x30 mL). All organic were combined and dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was purified by column chromatography using 10-20% ethyl acetate in n -heptane. The pure fraction were combined and concentrated under reduced pressure to obtain trioctyl 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [XX-5] (3.1 g, 4.39 mmol, yield 85.67%) as a colourless liquid.

[0954] ELSD: Purity 90.57 %, Calculated Mass for $\text{C}_{36}\text{H}_{65}\text{BrO}_8$ = 704.38, Observed = 705.30 (m/z , $\text{M}+\text{H}^+$).

JC-24-TL-001 (Compound 118):

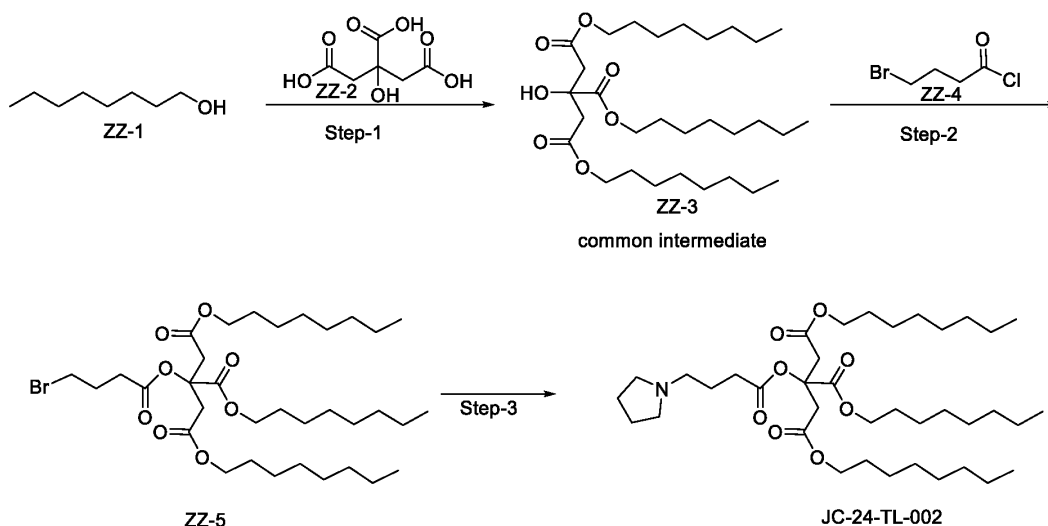


[0955] To a stirred solution of trioctyl 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [XX-5] (1.0 g, 1.42 mole, 1.0 eq.) in tetrahydrofuran (20 mL, 246 mmol), N,N-dimethylamine 2M solution [XX-6] (1.77 ml, 3.54 mole, 2.5 eq.) was successively added at room temperature. The resulting reaction mixture was stirred at 55°C temperature for 8 hrs. Progress of reaction was monitored by ELSD. ELSD data shows formation of desired compound. Reaction mixture was quenched over water- 100 ml. Stirred and separated the bottom organic layer. Removed the solvent under reduced pressure and resulting crude was purified over silica using 5-10%MeOH in DCM to get desired product as the HBR salt. The compound was dissolved in 30ml dry dichloromethane, followed by addition of Potassium carbonate (2.0 eq) to solution. The mixture was stirred for 90 minutes, then Filtered through 0.2 mm membrane filter. Recovered solvent under reduced pressure to get desired trioctyl 2-((6-(dimethylamino)hexanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-001] (0.330 g, Yield- 35.10%) as a yellow liquid.

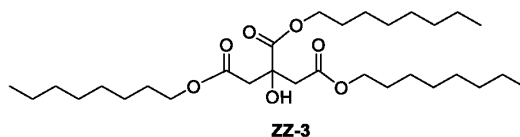
[0956] ¹H NMR (400 MHz, CDCl₃): δ 4.13 (t, *J* = 6.8 Hz, 2H), 4.07- 4.04 (m, 4H), 3.29 - 3.25 (d, *J* = 15.4 Hz, 2H), 3.20 – 3.16 (d, *J* = 15.4 Hz, 2H), 2.57 (m, 2H), 2.47 (s, 6H), 2.32 (t, *J* = 7.32 Hz, 2H), 1.70-1.54 (m, 10H), 1.38 - 1.26 (m, 32H), 0.87 (t, *J* = 6.52 Hz, 9H). ELSD: Purity 99.70 %, Calculated Mass for C₃₈H₇₁NO₈ = 669.52, Observed = 670.80 (*m/z*, M+H⁺). CAD Purity: 96.50 %.

Example 40J. Synthesis of JC-24-TL-002 (Compound 119)

Synthetic Protocol XX



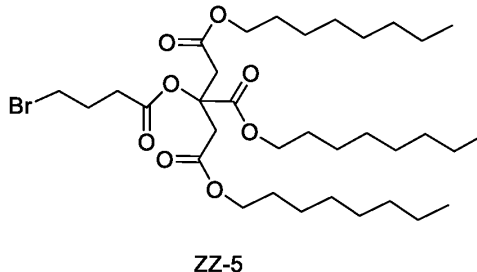
Intermediate [ZZ-3]:



[0957] To a solution of citric acid [ZZ-2] (25.0 g, 0.130 mol) and octan-1-ol [ZZ-1] (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol) was added, and the resulting mixture was stirred at 100 °C for 24 h. TLC (5% Ethyl acetate/hexanes) monitored the reaction progress. The reaction mixture was cooled to RT, diluted with water (500 mL), and extracted with ethyl acetate (500 mL x 3). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The crude was purified by column chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [ZZ-3] (40.0 g, Yield- 58.18 %) as a colourless liquid.

[0958] ^1H NMR (400 MHz, CDCl_3): δ 5.61 (s, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.4 Hz, 4H), 2.87 (d, J = 15.2 Hz, 2H), 2.72 (d, J = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 97.38 %, Calculated $\text{C}_{30}\text{H}_{56}\text{O}_7$ = 528.77, Observed = 529.2 (m/z , $\text{M}+\text{H}^+$).

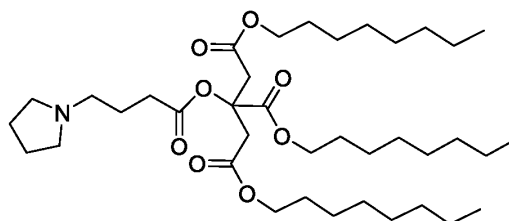
Intermediate [ZZ-5]:



[0959] To a stirred solution of trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [ZZ-3] (1g, 104 μmol) in dichloromethane (40 mL, 625 mmol) and Triethylamine (3.16 μL , 3 eq., 22.7 mmol) was added 4-bromobutyl chloride [ZZ-4] (2.81 g, 2 eq., 15.1 mmol) at 0 °C under nitrogen atmosphere. The resulting reaction mixture was allowed to stir at room temperature for 16 h. The progress of reaction was monitored by TLC & ELSD (SM was consumed). After completion, reaction mass was extracted with DCM (2x 200ml). The combined organic layer were dried over anhydrous sodium sulphate and concentrated under reduced pressure to get crude. The crude was then purified over silica (0-20% EtOAC/Hexane) to get the desired product trioctyl 2-((4-bromobutanoyl)oxy)propane-1,2,3-tricarboxylate [ZZ-5] (1.0 g, Yield-78%) as colourless liquid.

[0960] ^1H NMR (400 MHz, CDCl_3): δ 4.20 – 4.13(m, 2H), 4.11 – 4.09 (m, 6H), 3.36 – 3.33 (d, J = 15.20 Hz, 2H), 3.27 – 3.23 (d, J = 15.20 Hz, 2H), 2.54 (t, J = 7.2 Hz, 2H), 1.67- 1.61 (m, 6H), 1.29 (m, 32H), 0.90 (t, J = 6.8 Hz, 9H).

JC-24- TL-002 (Compound 119):



JC-24-TL-002

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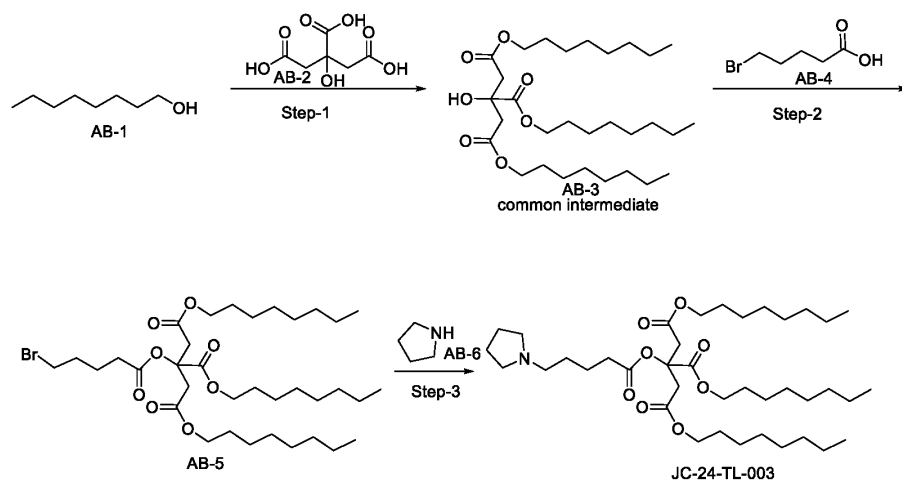
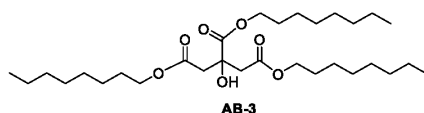
[0961] To a stirred solution of trioctyl 2-((4-bromobutanoyl)oxy)propane-1,2,3-tricarboxylate [ZZ-5] (1 g, 1.48 mmol) in tetrahydrofuran (20.4 mL, 251 mmol), pyrrolidine (262 mg, 2.5 eq., 3.69 mmol) was added at room temperature. After 10 min, the resulting reaction mixture allowed to stir at 55°C temperature for 16 h. The progress of reaction was monitor by ELSD. Reaction mixture was concentrated at high vacuum and purified by column chromatography by using (5-10%MeOH in DCM) to get trioctyl 2-((4-(pyrrolidin-1-yl)butanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-002] (150 mg, yield- 15.22%) as a yellow liquid.

[0962] ^1H NMR (400 MHz, CDCl_3): δ 4.13 (t, J = 6.76 Hz, 2H), 4.10 - 4.01 (m, 4H), 3.27 – 3.25 (d, J = 15.36 Hz, 2H), 3.18 – 3.14 (d, J = 15.36 Hz, 2H), 2.94 (brs, 4H), 2.42 (t, J = 6.56 Hz, 2H), 2.10-2.02 (m, 6H), 1.65-1.58 (m, 6H), 1.35-1.2 (m, 32H), 0.87 (t, J = 6.56 Hz, 9H). ELSD analysis: Purity 99.66 %, Calculated $\text{C}_{38}\text{H}_{69}\text{NO}_8$ = 667.50, Observed = 668.50 (m/z, $\text{M}+\text{H}^+$). CAD purity: 96.14 %.

15

Example 40K. Synthesis of JC-24-TL-003 (Compound 120)

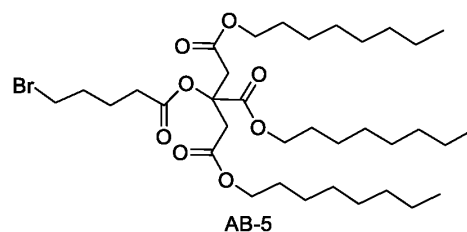
Synthetic Protocol ZZ

**Intermediate [AB-3]:**

[0963] To a solution of citric acid [AB-2] (25.0 g, 0.130 mol) and octan-1-ol [AB-1] (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol) was added, and the resulting mixture was stirred at 100 °C for 24 h. TLC (5% Ethyl acetate/hexanes) monitored the reaction progress. The reaction mixture was cool to RT, diluted with water (500 mL) and extracted with ethyl acetate (500 mL x 3). The organic layer dried over anhydrous sodium sulphate and evaporated under reduced pressure. The crude was purified by column chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [AB-3] (40.0 g, Yield- 58.18 %) as a colourless liquid.

[0964] ^1H NMR (400 MHz, CDCl_3): δ 5.61 (s, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.4 Hz, 4H), 2.87 (d, J = 15.2 Hz, 2H), 2.72 (d, J = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 97.38 %, Calculated $\text{C}_{30}\text{H}_{56}\text{O}_7$ = 528.77, Observed = 529.2 (m/z , $\text{M}+\text{H}^+$).

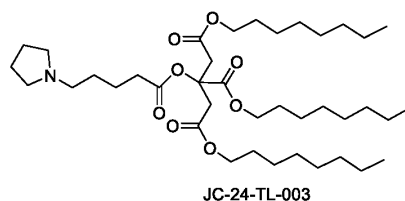
Intermediate [AB-5]:



[0965] To the stirred solution of 5-bromovaleric acid (4 g, 4 eq., 22.1 mmol) in dichloromethane (60 mL, 937 mmol), were added N,N-dimethyl-4-pyridylamine (1.35 g, 2 eq., 11 mmol) and EDC.HCl (4.24 g, 4 eq., 22.1 mmol) at room temperature under nitrogen atmosphere. After 10 minutes, trioctyl 2-hydroxy-1,2,3-propanetricarboxylate (2.92 g, 5.52 mmol) was added to the resulting reaction mass and allowed to stir at room temperature for 48 h. The progress of reaction was monitor by TLC. The reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (2x 30 mL). The combined organic layer was and dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude material was purified by silica gel flash column chromatography using 10-20% ethyl acetate in n-heptane. The pure fraction were combined and concentrated under reduced pressure to obtain trioctyl 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate **[AB-5]** (2.2 g, Yield 57.57%) as a colourless liquid.

[0966] ¹H NMR (400 MHz, CDCl₃): δ 4.15 (t, *J* = 6.8 Hz, 2H), 4.09- 4.06 (m, 4H), 3.32 - 3.28 (d, *J* = 15.2 Hz, 2H), 3.23 – 3.19 (d, *J* = 15.6 Hz, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 1.93- 1.89 (m, 2H), 1.88-1.75 (m, 2H), 1.67-1.57 (m, 8H), 1.33 - 1.28 (m, 30H), 0.87 (t, *J* = 7.2 Hz, 9H). ELSD analysis: Purity 91.45 %, Calculated C₃₅H₆₃BrO₈= 690.37, Observed = 691.25 (m/z, M+H⁺).

JC-24- TL-003 (Compound 120):



[0967] To a stirred solution of trioctyl 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate **[AB-5]** (1 g, 1.45 mmol) in tetrahydrofuran (20 mL, 246 mmol), pyrrolidine **[AB-6]** (257 mg, 2.5 eq., 3.61 mmol) was added at room temperature and stirred for 10 minute. The resulting reaction mixture was allowed to stir at 55 °C temperature for 8 h. Reaction progress was monitored by ELSD. Reaction mixture was concentrated under reduced pressure and purified by silica gel flash

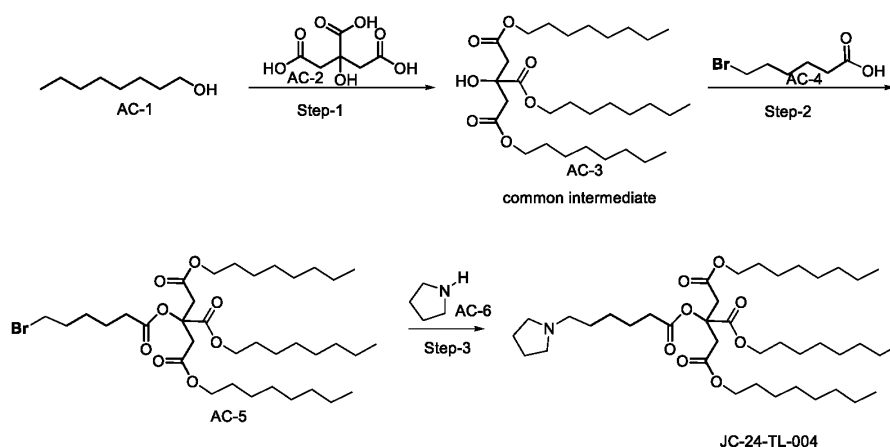
column chromatography using 5-10% MeOH in DCM to afford HBR salt of desired product.

Obtained product salt taken in dry dichloromethane (3 ml), and added Potassium carbonate (2.0 eq) to the resulting solution and stirred for 90 minute. The resulting solution filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to get trioctyl 2-((5-
 5 (pyrrolidin-1-yl)pentanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-003] (0.250 g, yield-
 25.35%) as a yellow liquid.

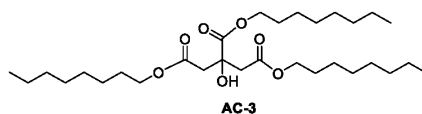
[0968] ^1H NMR (400 MHz, CDCl_3): δ 4.13 (t, J = 6.8 Hz, 2H), 4.07- 4.04 (m, 4H), 3.29 - 3.25 (d, J = 15.4 Hz, 2H), 3.20 – 3.16 (d, J = 15.4 Hz, 2H), 2.73 (brs, 4H), 2.35 (t, J = 7.12 Hz, 2H), 1.94 (brs, 4H), 1.79-1.57 (m, 10H), 1.40 - 1.16 (m, 32H), 0.87 (t, J = 7.04 Hz, 9H). ELSD analysis: Purity 99.62 %,
 10 Calculated $\text{C}_{39}\text{H}_{71}\text{NO}_8$ = 681.52, Observed = 682.45 (m/z , $\text{M}+\text{H}^+$). CAD Purity: 97.94 %.

Example 40L. Synthesis of JC-24-TL-004 (Compound 121)

Synthetic Protocol AB



Intermediate [AC-3]:

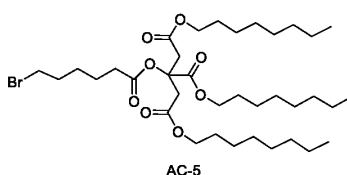


[0969] To a solution of citric acid [AC-2] (25.0 g, 0.130 mol) and octan-1-ol [AC-1] (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol,) was added, and the resulting mixture was stirred at 100 °C for 24 h. The reaction progress was monitored by TLC (5% Ethyl acetate/hexanes). The reaction mixture was cooled to RT, diluted with water (500 mL) and
 20 extracted with ethyl acetate (500 mL x 3). The organic layer dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude was purified by column

chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [AC-3] (40.0 g, Yield- 58.18 %) as a colourless liquid.

- [0970] ^1H NMR (400 MHz, CDCl_3): δ 5.61 (s, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.4 Hz, 4H), 2.87 (d, J = 15.2 Hz, 2H), 2.72 (d, J = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 97.38 %, Calculated $\text{C}_{30}\text{H}_{56}\text{O}_7$ = 528.77, Observed = 529.2 (m/z , $\text{M}+\text{H}^+$).

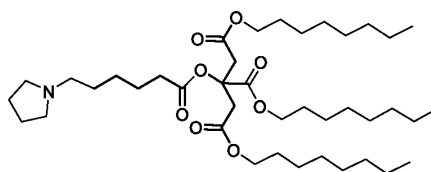
Intermediate [AC-5]:



- 10 [0971] To a stirred solution of 6-bromohexanoic acid [AC-4] (4.0 g, 4 eq., 20.5 mmol) in dichloromethane (50 mL), N,N -dimethyl-4-pyridylamine (2.5 g, 20.5 mmol, 4 eq.,) and EDC.HCl (3.93 g, 20.5 mmol, 4 eq.,) were added successively at room temperature. After 10 minutes, trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [AC-3] (2.64 g, 5.13 mmol) was added at room temperature and the resulting reaction mixture was stirred at room temperature for 48 h.
- 15 Reaction progress was monitor by TLC/ELSD until consumption of the starting material. The resulting reaction mixture was quenched with water (100 mL) and extracted with dichloromethane (2x30 mL). All organic were combined and dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was purified by column chromatography using 10-20% ethyl acetate in n -heptane. The pure fraction were combined and concentrated
- 20 under reduced pressure to obtain trioctyl 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AC-5] (3.1 g, 4.39 mmol, yield 85.67%) as a colourless liquid.

[0972] ELSD: Purity 90.57 %, Calculated Mass for $\text{C}_{36}\text{H}_{65}\text{BrO}_8$ = 704.38, Observed = 705.30 (m/z , $\text{M}+\text{H}^+$).

JC-24- TL-004 (Compound 121):



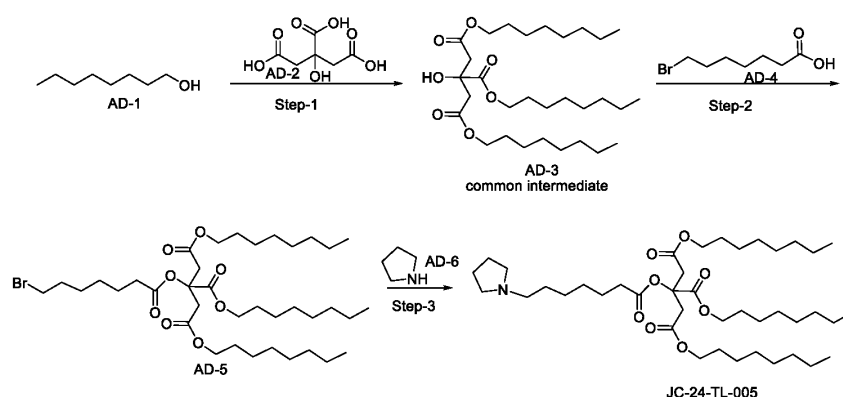
JC-24-TL-004

[0973] To a stir solution of trioctyl 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AC-5] (1 g, 1.45 mmol) in tetrahydrofuran (20 mL, 246 mmol), pyrrolidine [AC-6] (257 mg, 2.5 eq., 3.61 mmol) was added at room temperature. After 10 min the resulting reaction mixture was allowed to stir at 55°C temperature for 16 h. The progress of reaction monitored by ELSD. Reaction mixture was concentrated under reduced pressure and purified by silica gel flash column chromatography (5-10%MeOH in DCM) to afford HBR salt of desired product. The product salt was taken up in dry dichloromethane (5 ml), and added Potassium carbonate (2.0 eq) to the resulting solution and stirred for 90 minute. The resulting solution filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to get trioctyl 2-((6-(pyrrolidin-1-yl)hexanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24- TL-004] (0.110 g, Yield 11.13%) as a yellow liquid.

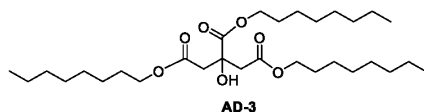
[0974] ^1H NMR (400 MHz, CDCl_3): δ 4.13 (t, J = 6.8 Hz, 2H), 4.08 - 4.03 (m, 4H), 3.29 - 3.25 (d, J = 15.4 Hz, 2H), 3.20 - 3.16 (d, J = 15.4 Hz, 2H), 2.75 (brs, 4H), 2.32 (t, J = 7.28 Hz, 2H), 1.98 (brs, 4H), 1.74 (brs, 2H), 1.66-1.57 (m, 8H), 1.37-1.26 (m, 34H), 0.87 (t, J = 7.0 Hz, 9H). ELSD: Purity 99.95 %, Calculated Mass for $\text{C}_{40}\text{H}_{73}\text{NO}_8$ = 695.53, Observed = 696.35 (m/z , $\text{M}+\text{H}^+$). Cad Purity: 95.21 %.

Example 40M. Synthesis of JC-24-TL-005 (Compound 122)

Synthetic Protocol AC



20 **Intermediate [AD-3]:**



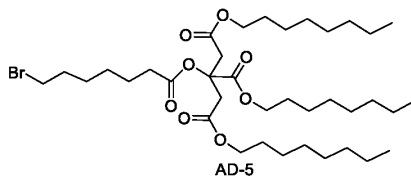
[0975] To a solution of citric acid [AD-2] (25.0 g, 0.130 mol) and octan-1-ol [AD-1] (67.7 g, 0.52 mol) in toluene (500 mL), PTSA (13.0 g, 0.065 mol,) was added, and the resulting mixture was stirred at 100 °C for 24 h. The reaction progress was monitored by TLC (5% Ethyl

5 acetate/hexanes). The reaction mixture was cooled to RT, diluted with water (500 mL) and extracted with ethyl acetate (500 mL x 3). The organic layer dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude was purified by column chromatography using 60-120 mesh silica and eluted with 5% ethyl acetate in hexane to give trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [AD-3] (40.0 g, Yield- 58.18 %) as a colourless liquid.

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[0976] ^1H NMR (400 MHz, CDCl_3): δ 5.61 (s, 1H), 4.01 (t, J = 6.8 Hz, 2H), 3.96 (t, J = 6.4 Hz, 4H), 2.87 (d, J = 15.2 Hz, 2H), 2.72 (d, J = 15.2 Hz, 2H), 1.57-1.49 (m, 6H), 1.24 (brs, 30H), 0.85 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 97.38 %, Calculated $\text{C}_{30}\text{H}_{56}\text{O}_7$ = 528.77, Observed = 529.2 (m/z , $\text{M}+\text{H}^+$).

15 **Intermediate [AD-5]:**



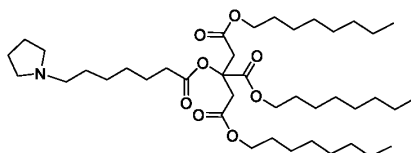
[0977] To the stirred solution of 7-bromoheptanoic acid [AD-4] (4 g, 4 eq., 19.1 mmol) in dichloromethane (49.6 mL), were added N,N-dimethyl-4-pyridylamine (1.17 g, 2 eq., 9.57 mmol) and EDC.HCl (4.58 g, 5 eq., 23.9 mmol) successively at room temperature. After 10 minute,

20 trioctyl 2-hydroxypropane-1,2,3-tricarboxylate [AD-3] (2.53 g, 4.78 mmol) was then added, and the resulting reaction mixture was stirred at room temperature for 48 h. The progress of reaction was monitor by TLC. The resulting reaction mixture quenched with water (100 mL) and extracted with dichloromethane (2x100 mL). The resulting organic layer dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was purified by silica gel flash column

25 chromatography using 10-20% ethyl acetate in n-heptane. The pure fraction were combined and concentrated under reduced pressure to obtain trioctyl 2-((7-bromoheptanoyl)oxy)propane-1,2,3-tricarboxylate [AD-5] (2.3 g, yield 66.81%) as a colourless liquid.

[0978] ELSD: Purity 95.50 %, Calculated Mass for $C_{37}H_{67}BrO_8$ = 718.40, Observed = 719.35 (m/z , $M+H^+$).

JC-24- TL-005 (Compound 122):



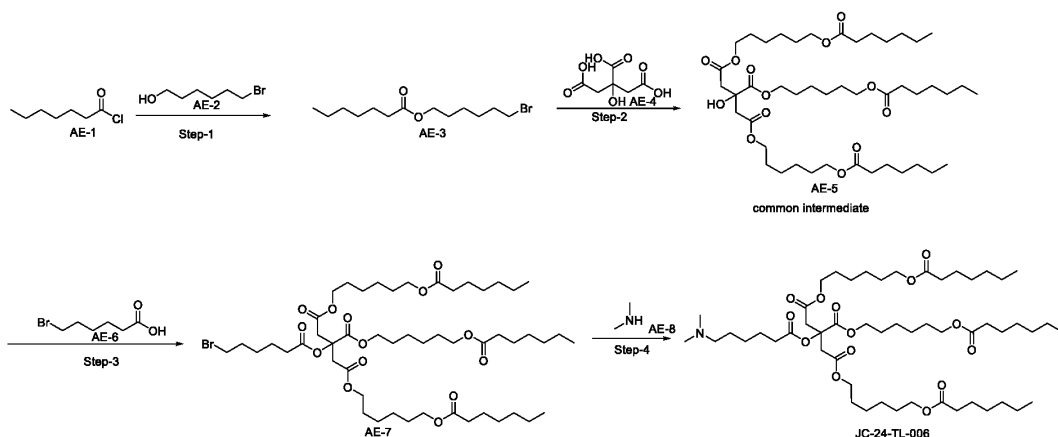
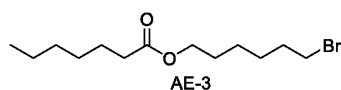
JC-24-TL-005

5 **[0979]** To a stirred solution of trioctyl 2-((7-bromoheptanoyl)oxy)propane-1,2,3-tricarboxylate [AD-5] (1 g, 1.39 mmol) in tetrahydrofuran (2.8 mL, 34.3 mmol), pyrrolidine [AD-6] (247 mg, 2.5 eq., 3.47 mmol) was added at room temperature and stirred for 10 minutes. The resulting reaction mixture allowed to stir at 55 °C temperature for 8 h. Reaction progress was monitor by ELSD. Reaction mixture was concentrated under reduced pressure and purified by silica gel flash
10 column chromatography using 5-10% MeOH in DCM to afford HBR salt of desired product. The product salt was taken up in dry dichloromethane (3 ml), added Potassium carbonate (2.0 eq) to the resulting solution, and stirred for 90 minute. The resulting solution was then filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to get trioctyl 2-((7-(pyrrolidin-1-yl)heptanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-005] (0.175 g, yield-
15 17.73%) as a yellow liquid.

[0980] ¹H NMR (400 MHz, CDCl₃): δ 4.11 (t, *J* = 6.8 Hz, 2H), 4.07- 4.01 (m, 4H), 3.30 - 3.26 (d, *J* = 15.4 Hz, 2H), 3.21 – 3.17 (d, *J* = 15.4 Hz, 2H), 2.92-2.66 (m, 6H), 2.30 (t, *J* = 7.48 Hz, 2H), 1.93 (brs, 4H), 1.74-1.53 (m, 10H), 1.36-1.20 (m, 34H), 0.87 (t, *J* = 7.0 Hz, 9H). ELSD: Purity 99.55 %, Calculated Mass for C₄₁H₇₅NO₈ = 709.55. Observed = 710.40 (*m/z*, M+H⁺). CAD purity: 97.96 %.

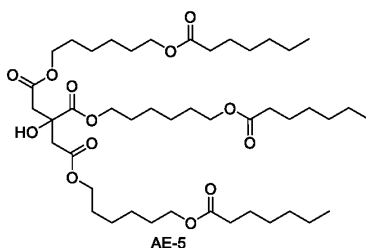
20 **Example 40N. Synthesis of JC-24-TL-006 (Compound 112)**

Synthetic Protocol AD

**Intermediate [AE-3]:**

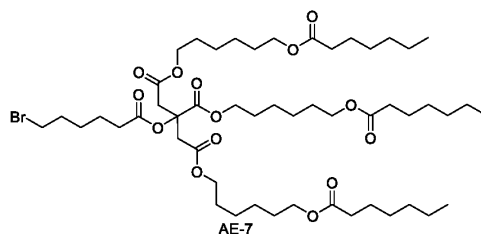
[0981] To a stirred solution of 6-bromo-1-hexanol [AE-2] (25 g, 138 mmol) in dichloromethane (237 mL, 3.71 mol) was added triethylamine (48.1 mL, 2.5 eq., 345 mmol). Reaction mass was cooled to 0 °C, and heptanoyl chloride [AE-1] (20.5 g, 138 mmol) was added as drop wise. Reaction mass allowed to stir at RT for 16 h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction mass was diluted with water (500 ml) and was extracted with dichloromethane (100 ml). The combined organic layer washed with aq. sodium bicarbonate solution (1000 ml), dried over sodium sulphate, and concentrated under reduced pressure. The crude was purified over silica using 5-10% ethyl acetate to give product 6-bromohexyl heptanoate [AE-3] (30 g, Yield- 74 %) as colourless liquid.

[0982] ^1H NMR (400 MHz, CDCl_3): δ 4.05 (t, J = 6.6 Hz, 2H), 3.39 (t, J = 2.7 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.90-1.82 (m, 2H), 1.67-1.57 (m, 4H), 1.50-1.35 (m, 4H), 1.35-1.32 (m, 6H), 0.87 (t, J = 6.4 Hz, 3H).

Intermediate [AE-5]:

[0983] To a stirred solution of 6-bromohexyl heptanoate [AE-3] (50.4 g, 3.3 eq., 172 mmol) and 2-hydroxy-1,2,3-propanetricarboxylic acid [AE-4] (10 g, 52 mmol) in dimethylformamide (0.5 L, 6.46 mol) was added TEA (43.9 mL, 6 eq., 312 mmol). The reaction mixture was heated to 50 °C for 16h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the SM, the reaction mixture was diluted with water (500 ml) and extracted with diethyl ether (3x 100 ml). The separated organic layer was dried over Na₂SO₄ and concentrated under reduce pressure to obtained crude product. Resulting crude was purified over silica using 20% ethyl acetate in n-hexane to obtained tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AE-5] (28.0 g, Yield- 65 %) as colour less liquid. ELSD analysis: Purity 99.88 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.60 (m/z, M+H⁺).

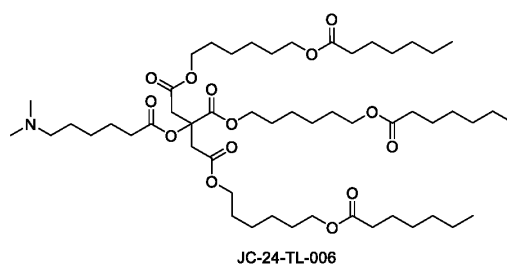
Intermediate [AE-7]:



[0984] To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AE-5] (4 g, 4.82 mmol) and 6-bromohexanoic acid [AE-6] (3.76 g, 4 eq., 19.3 mmol) in dichloromethane (50 mL, 781 mmol) was added EDC.HCl (3.7 g, 4 eq., 19.3 mmol) followed by addition of N,N-dimethyl-4-pyridylamine (1.18 g, 2 eq., 9.65 mmol). Reaction mixture was stirred for 16 h at room temperature, wherein ELSD/TLC confirmed consumption of the SM. Water (500ml) added to the reaction mixture and extracted with DCM (3x100 ml). The separated organic layer was dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Resulting crude was purified over silica using 5% ethyl acetate in n-hexane to obtained pure product tris(6-(heptanoyloxy)hexyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AE-7] (2.9 g, yield 57.94%) as colour less liquid.

[0985] ELSD analysis: Purity 99.40 %, Calculated C₅₁H₈₉BrO₁₄ = 1004.54, Observed = 1005.50 (m/z, M+H⁺).

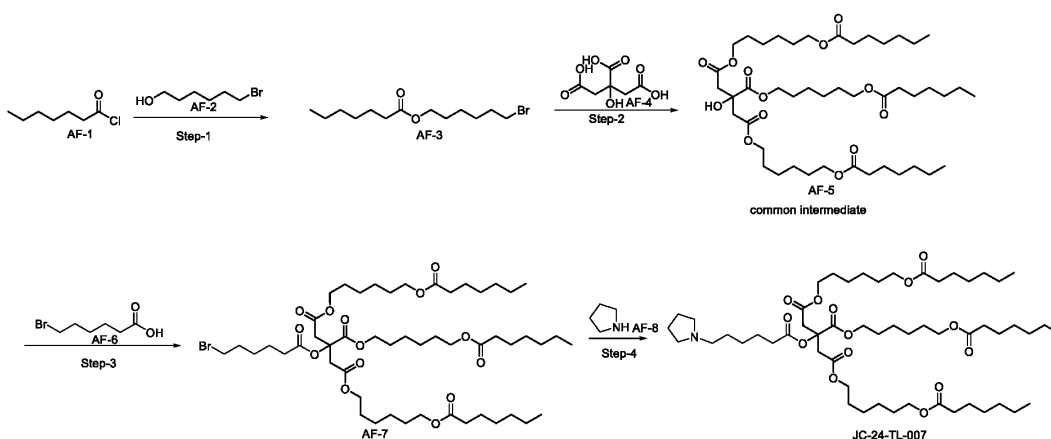
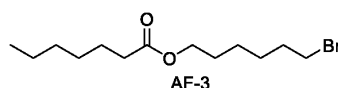
JC-24-TL-006 (Compound 112):



[0986] To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AE-7] (1 g, 994 μmol) in tetrahydrofuran (10 mL, 123 mmol) was added dimethylamine (1.24 mL, 2M solution in THF) [AE-8] (112 mg, 2.5 eq., 2.48 mmol). Reaction mass was then heated to 55 °C for 16h, with progress of the reaction being monitored by ELSD/TLC. Reaction mass cooled to room temperature and the concentrated under reduce pressure to obtained crude product. The resulting crude material was purified over silica using flash chromatography and 5% MeOH in DCM to obtain product as HBR salt. The salt was dissolved in dry dichloromethane (3 ml), potassium carbonate (2.0 eq) added to the solution, and allow to stir for 90 minutes. The solution was filtered through 0.2 mm membrane filter. The solvent was removed under reduced pressure to obtain tris(6-(heptanoyloxy)hexyl) 2-((6-(dimethylamino)hexanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-006] (450 mg, Yield-46.66%) as light yellow liquid.

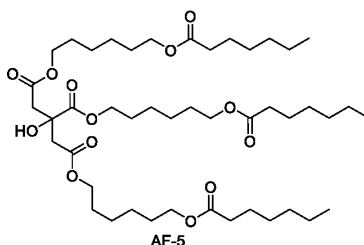
[0987] ^1H NMR (400 MHz, CDCl_3): δ 4.14 (t, J = 6.72 Hz, 2H), 4.08 - 4.03 (m, 10H), 3.29 - 3.25 (d, J = 15.48 Hz, 2H), 3.20-3.16 (d, J = 15.48 Hz, 2H), 2.63 (brs, 2H), 2.51 (brs, 4H), 2.34-2.26 (m, 8H), 1.72 – 1.57 (m, 22H), 1.42-1.34 (m, 15 H), 1.33-1.24 (m, 19H), 0.89 (t, J = 7.0 Hz, 9H). ELSD analysis: Purity 99.88 %, Calculated $\text{C}_{53}\text{H}_{95}\text{NO}_{14}$ = 969.68, Observed = 970.50 (m/z , $\text{M}+\text{H}^+$). CAD purity = 95.65 %.

Example 400. Synthesis of JC-24-TL-007 (Compound 113)

**Intermediate [AF-3]:**

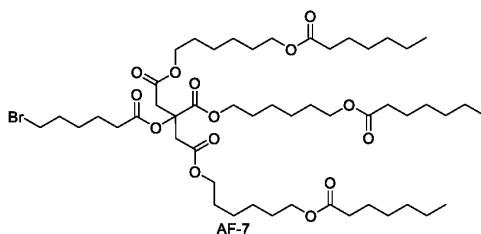
[0988] To a stirred solution of 6-bromo-1-hexanol [AF-2] (25 g, 138 mmol) in dichloromethane (237 mL, 3.71 mol) was added triethylamine (48.1 mL, 2.5 eq., 345 mmol). Reaction mass was cooled to 0 °C, and heptanoyl chloride [AF-1] (20.5 g, 138 mmol) was added as drop wise. Reaction mass allowed to stir at RT for 16 h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction mass was diluted with water (500 ml) and was extracted with dichloromethane (100 ml). The combined organic layer washed with aq. sodium bicarbonate solution (1000 ml), dried over sodium sulphate, and concentrated under reduced pressure. The crude was purified over silica using 5-10% ethyl acetate to give product 6-bromohexyl heptanoate [AF-3] (30 g, Yield- 74 %) as colourless liquid.

[0989] ^1H NMR (400 MHz, CDCl_3): δ 4.05 (t, J = 6.6 Hz, 2H), 3.39 (t, J = 2.7 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.90-1.82 (m, 2H), 1.67-1.57 (m, 4H), 1.50-1.35 (m, 4H), 1.35-1.32 (m, 6H), 0.87 (t, J = 6.4 Hz, 3H).

Intermediate [AF-5]:

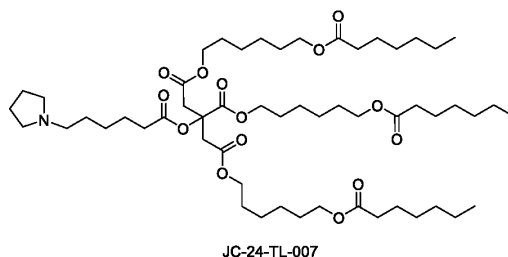
[0990] To a stirred solution of 6-bromohexyl heptanoate [AF-3] (50.4 g, 3.3 eq., 172 mmol) and 2-hydroxy-1,2,3-propanetricarboxylic acid [AF-4] (10 g, 52 mmol) in dimethylformamide (0.5 L, 6.46 mol) was added TEA (43.9 mL, 6 eq., 312 mmol). Reaction mas was heated to 50 °C for 16h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the SM, the reaction mixture was diluted with water (500 ml) and extracted with diethyl ether (3x 100 ml). The separated organic layer was dried over Na₂SO₄ and concentrated under reduce pressure to obtained crude product. Resulting crude was purified over silica using 20% ethyl acetate in n-hexane to obtained tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AF-5] (28.0 g, Yield- 65 %) as colour less liquid. ELSD analysis: Purity 99.88 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.60 (m/z, M+H⁺).

Intermediate [AF-7]:



[0991] To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AF-5] (4 g, 4.82 mmol) and 6-bromohexanoic acid [AF-6] (3.76 g, 4 eq., 19.3 mmol) in dichloromethane (50 mL, 781 mmol) was added EDC.HCl (3.7 g, 4 eq., 19.3 mmol) followed by addition of N,N-dimethyl-4-pyridylamine (1.18 g, 2 eq., 9.65 mmol). Reaction mixture was stirred for 16 h at room temperature, wherein ELSD/TLC confirmed consumption of the SM. Water (500ml) added to the reaction mixture and extracted with DCM (3x100 ml). The separated organic layer was dried over Na₂SO₄, filtered, and concentrated under reduce pressure. Resulting crude was purified over silica using 5% ethyl acetate in n-hexane to obtained pure product tris(6-(heptanoyloxy)hexyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AF-7] (2.9 g, yield 57.94%) as colour less liquid. ELSD analysis: Purity 99.40 %, Calculated C₅₁H₈₉BrO₁₄ = 1004.54, Observed = 1005.50 (m/z, M+H⁺).

JC-24-TL-007 (Compound 113):

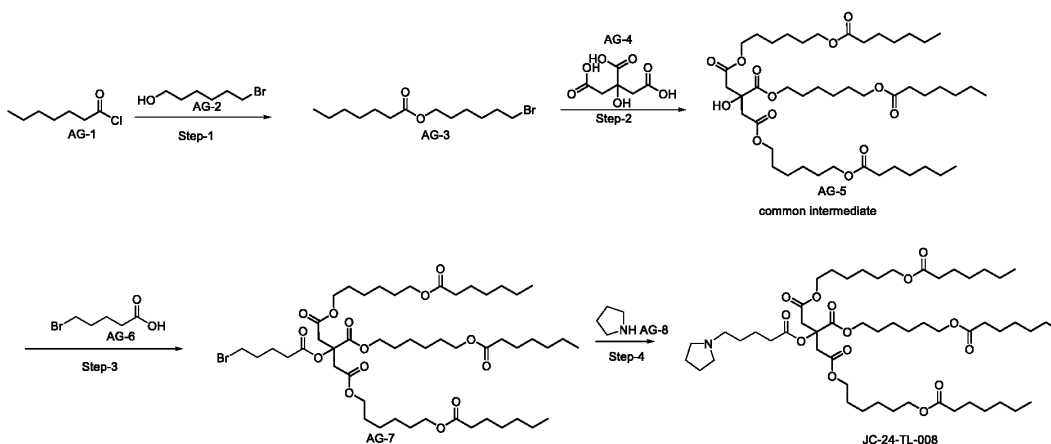


- [0992]** To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AF-7] (1 g, 994 μmol) in tetrahydrofuran (10 mL, 123 mmol) was added pyrrolidine [AF-8] (177 mg, 2.5 eq., 2.48 mmol). Reaction mass was then heated to 55 °C for 16h, with progress of the reaction being monitored by ELSD/TLC. Reaction mass was cooled to room temperature and concentrated under reduce pressure to obtained crude product. The resulting crude product was purified over silica using flash chromatography and 5% MeOH in DCM to obtain product as HBR salt. The salt was dissolved in dry dichloromethane (3 ml), potassium carbonate (2.0 eq) added to the solution, and allow to stir for 90 minutes. The solution was filtered through 0.2 mm membrane filter. The solvent was removed under reduced pressure to obtain tris(6-(heptanoyloxy)hexyl) 2-((6-(pyrrolidin-1-yl)hexanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-007] (330 mg, yield- 33.32%) as light yellow liquid.

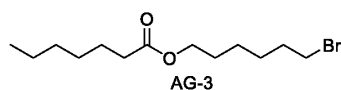
- [0993]** ^1H NMR (400 MHz, CDCl_3): δ 4.13 (t, J = 6.72 Hz, 2H), 4.09 - 4.02 (m, 10H), 3.29 - 3.25 (d, J = 15.48 Hz, 2H), 3.20 - 3.16 (d, J = 15.48 Hz, 2H), 2.71 (brs, 4H), 2.33-2.26 (m, 8H), 1.95 (s, 4H), 1.78-1.56 (m, 24H), 1.43-1.36 (m, 14 H), 1.32-1.23 (m, 18H), 0.89 (t, J = 7.0 Hz, 9H). ELSD analysis: Purity 99.85 %, Calculated $\text{C}_{55}\text{H}_{97}\text{NO}_{14}$ = 995.69, Observed = 996.85 (m/z , $\text{M}+\text{H}^+$). Cad purity = 93.84 %.

Example 40P. Synthesis of JC-24-TL-008 (Compound 114)

Synthetic Protocol AF



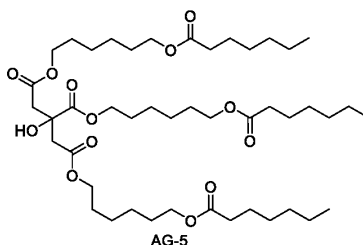
Intermediate [AG-3]:



[0994] To a stirred solution of 6-bromo-1-hexanol [AG-2] (25 g, 138 mmol) in dichloromethane (237 mL, 3.71 mol) was added triethylamine (48.1 mL, 2.5 eq., 345 mmol). Reaction mass was cooled to 0 °C, and heptanoyl chloride [AG-1] (20.5 g, 138 mmol) was added as drop wise. Reaction mass allowed to stir at RT for 16 h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction mass was diluted with water (500 ml) and was extracted with dichloromethane (100 ml). The combined organic layer washed with aq. sodium bicarbonate solution (1000 ml), dried over sodium sulphate, and concentrated under reduced pressure. The crude was purified over silica using 5-10% ethyl acetate to give product 6-bromohexyl heptanoate [AG-3] (30 g, Yield- 74 %) as colourless liquid.

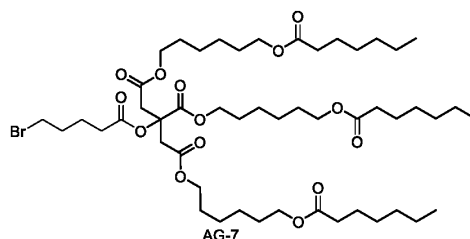
[0995] ¹H NMR (400 MHz, CDCl₃): δ 4.05 (t, *J* = 6.6 Hz, 2H), 3.39 (t, *J* = 2.7 Hz, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.90-1.82 (m, 2H), 1.67-1.57 (m, 4H), 1.50-1.35 (m, 4H), 1.35-1.32 (m, 6H), 0.87 (t, *J* = 6.4 Hz, 3H).

Intermediate [AG-5]:



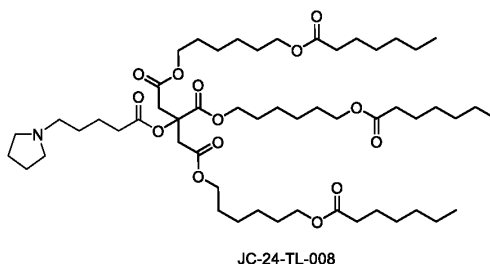
[0996] To a stirred solution of 6-bromohexyl heptanoate [AG-3] (50.4 g, 3.3 eq., 172 mmol) and 2-hydroxy-1,2,3-propanetricarboxylic acid [AG-4] (10 g, 52 mmol) in dimethylformamide (0.5 L, 6.46 mol) was added TEA (43.9 mL, 6 eq., 312 mmol). Reaction mas was heated to 50 °C for 16h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the SM, the reaction mixture was diluted with water (500 ml) and extracted with diethyl ether (3x 100 ml). The separated organic layer was dried over Na₂SO₄ and concentrated under reduce pressure to obtained crude product. Resulting crude was purified over silica using 20% ethyl acetate in n-hexane to obtained tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AG-5] (28.0 g, Yield- 65 %) as colour less liquid. ELSD analysis: Purity 99.88 %, Calculated C₄₅H₈₀O₁₃ = 828.56, Observed = 829.60 (m/z, M+H⁺).

Intermediate [AG-7]:



[0997] To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-hydroxypropane-1,2,3-tricarboxylate [AG-5] (2 g, 2.41 mmol) and 5-bromovaleric acid [AG-6] (1.75 g, 4 eq., 9.65 mmol) in dichloromethane (25 mL, 390 mmol), were added EDC.HCl (1.85 g, 4 eq., 9.65 mmol) followed by N,N-dimethyl-4-pyridylamine (589 mg, 2 eq., 4.82 mmol). Reaction mixture was stirred for 16h at room temperature, wherein ELSD/TLC confirmed consumption of the SM. Reaction mixture diluted with water (500 ml) and extracted with DCM (3x100 ml). The separated organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Resulting crude product was purified over silica using 5% ethyl acetate in n-hexane to obtained tris(6-(heptanoyloxy)hexyl) 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate [AG-7] (720 mg, yield- 30 %) as colourless liquid. ELSD analysis: Purity 99.20 %, Calculated C₅₀H₈₇BrO₁₄= 990.53, Observed = 991.40 (m/z, M+H⁺).

JC-24-TL-008 (Compound 114):

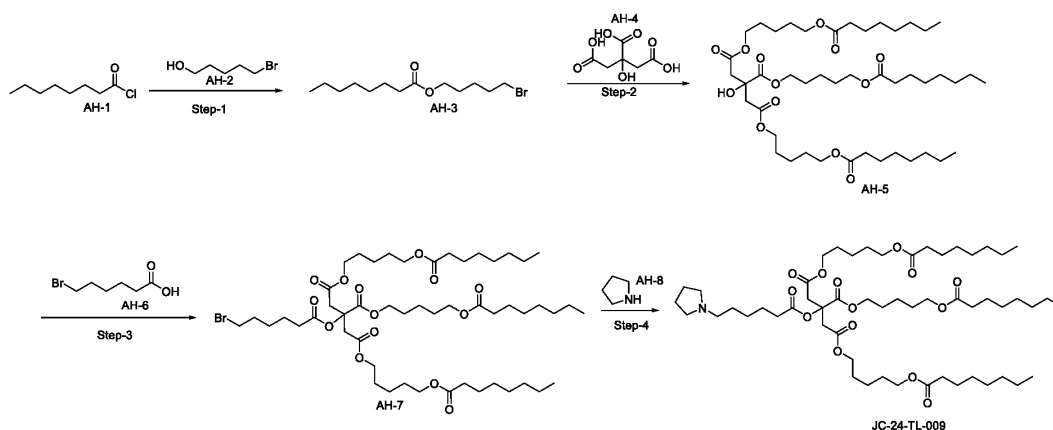
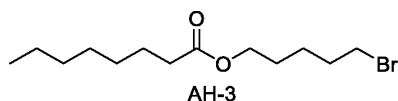


[0998] To a stirred solution of tris(6-(heptanoyloxy)hexyl) 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate [AG-7] (720 mg, 726 μmol) in tetrahydrofuran (10 mL, 123 mmol) was added pyrrolidine (103 mg, 2 eq., 1.45 mmol). Reaction mass was then heated to 55 °C for 16h, with progress of the reaction being monitored by ELSD/TLC. Reaction mass was cooled to room temperature and distilled out solvent to get crude product. The resulting crude material was purified over silica using flash chromatography (5% MeOH in DCM) to obtain product as HBR salt. The salt was dissolved in dry dichloromethane (3 ml), potassium carbonate (2.0 eq) added to the solution, and allow to stir for 90 minutes. After this time, the solution was filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to obtain tris(6-(heptanoyloxy)hexyl) 2-((5-(pyrrolidin-1-yl)pentanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-008] (220 mg, yield- 30.86 %) as colourless liquid.

[0999] $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 4.14 (t, J = 6.68 Hz, 2H), 4.09-4.03 (m, 10H), 3.28-3.24 (d, J = 15.44 Hz, 2H), 3.19-3.15 (d, J = 15.44 Hz, 2H), 2.93 (brs, 4H), 2.36 (t, J = 7.0 Hz, 2H), 2.28 (t, J = 7.52 Hz, 6H), 2.05 (s, 4H), 1.69-1.57 (m, 24H), 1.42-1.36 (m, 12 H), 1.29-1.25 (m, 18H), 0.87 (t, J = 7.0 Hz, 9H). ELSD analysis: Purity 99.85 %, Calculated $\text{C}_{54}\text{H}_{95}\text{NO}_{14}$ = 981.68, Observed = 982.85 (m/z , $\text{M}+\text{H}^+$). CAD Purity = 93.90 %.

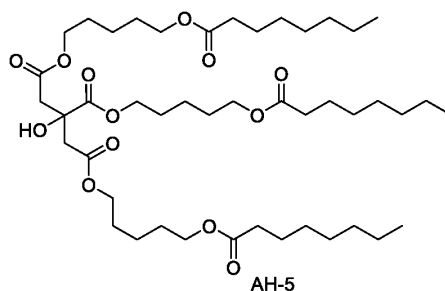
Example 40Q. Synthesis of JC-24-TL-009 (Compound 115)

Synthetic Protocol AG

**Intermediate [AH-3]:**

- 5 **[01000]** To a stirred solution of 5-bromo-1-pentanol [AH--2] (25 g, 150 mmol) in dichloromethane (257 mL, 4.02 mol) was added triethylamine (52.2 mL, 2.5 eq., 374 mmol). Reaction mass was cooled to 0 °C and octanoyl chloride [AH-1] (25.5 mL, 150 mmol) was added drop wise. Reaction mass allowed to stir at RT for 16 h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction mass was diluted
- 10 with water (500 ml) and extracted with dichloromethane (2x100 ml). Combined organic layer was washed with sodium bicarbonate solution (1000 ml). Organic layer was dried over sodium sulphate, filtered, and concentrated under reduced pressure. The crude was purified over silica get by using flash chromatography and 5-10% ethyl acetate to give 5-bromopentyl octanoate [AH-3] (31 g, yield 71.26 %) as colourless liquid.
- 15 **[01001] Note:** Two batches of 25.0 g (total 50 g) were performed yielding total 62 g of desired product.
- [01002]** ¹H NMR (400 MHz, CDCl₃): 4.07 (t, *J* = 6.52 Hz, 2H), 3.40 (t, *J* = 6.72Hz, 2H), 2.31-2.27 (t, *J* = 2.68 Hz, 2H), 1.92 - 1.85 (m, 2H), 1.69 – 1.59 (m, 4H), 1.54 – 1.43 (m, 2H), 1.34-1.22 (m, 8H), 0.87 (t, *J* = 6.76 Hz, 3H).

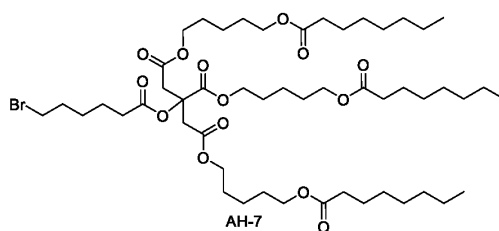
20 **Intermediate [AH-5]:**



[01003] To a stirred solution of 5-bromopentyl octanoate [AH-3] (61.1 g, 3.8 eq., 187 mmol) and 2-hydroxy-1,2,3-propanetricarboxylic acid [AH-4] (9.6 g, 50 mmol) in dimethylformamide (480 mL, 6.2 mol) was added TEA (69.6 mL, 10 eq., 0.5 mol). The reaction mixture was stirred for 16 h at 60 °C, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction was cooled to RT and then quenched with water (2000 mL) and extracted with diethyl ether (2x 500 mL). The organic layer dried over sodium sulphate, filtered, and concentrated under reduced pressure. Resulting crude was purified over silica using 20% ethyl acetate in n-hexane to obtained tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [AH-5] (21 g, yield-50.69%) as light yellow colour liquid.

[01004] ^1H NMR (400 MHz, CDCl_3): 4.21 (t, J = 6.68 Hz, 2H), 4.12-4.03 (m, 10H), 2.89- 2.85 (d, J = 15.50 Hz, 2H), 2.80-2.76 (d, J = 15.6 Hz, 2H), 2.30 (t, J = 6.4 Hz, 6H), 1.75 -1.56 (m, 18H), 1.45 -1.37 (m, 6H), 1.31-1.26 (m, 22H), 0.87 (t, J = 9.0 Hz, 9H).

Intermediate [AH-7]:



15

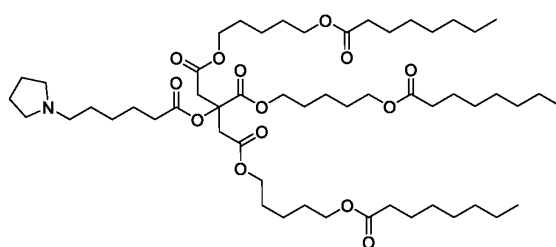
[01005] To a stirred solution of 6-bromohexanoic acid [AH-6] (1.32 g, 4 eq., 6.75 mmol) in dichloromethane (17.5 mL, 273 mmol), were added N,N-dimethyl-4-pyridylamine (413 mg, 2 eq., 3.38 mmol) and EDC.HCl (1.62 g, 5 eq., 8.44 mmol) at room temperature and stirred for 10 minute at same temperature. After this time, tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [AH-5] (1.4 g, 1.69 mmol) was added, and the resulting reaction mixture allowed to stir at room temperature for 36h, with progress of reaction being monitor by ELSD. The reaction mixture was quench with water (50 mL) and extracted with dichloromethane (2x 30 mL). The

20

combined organic layer dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The resulting crude material purified by using silica gel flash column chromatography (5-10% ethyl acetate in heptane). The pure fraction were combined and concentrated under reduced pressure to obtained tris(5-(octanoyloxy)pentyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AH-7] (0.8 g, yield 47.09%) as a colourless liquid.

[01006] ELSD analysis: Purity 99.87 %, Calculated $\text{C}_{51}\text{H}_{89}\text{BrO}_{14}$ = 1006.16, Observed = 1007.50 (m/z , $\text{M}+\text{H}^+$).

JC-24-TL-009 (Compound 115):



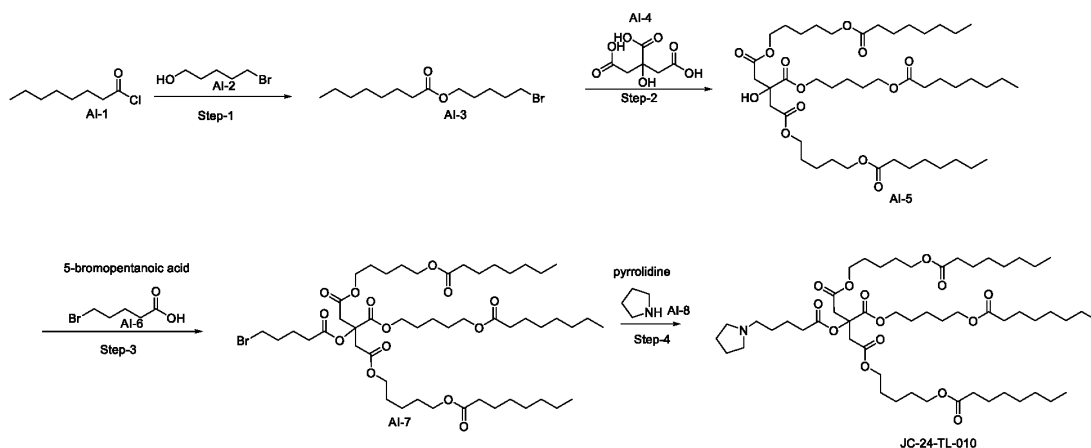
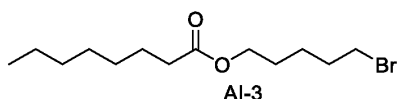
JC-24-TL-009

- [01007]** To a stirred solution of tris(5-(octanoyloxy)pentyl) 2-((6-bromohexanoyl)oxy)propane-1,2,3-tricarboxylate [AH-7] (0.8 g, 795 μmol) in tetrahydrofuran (10 mL, 123 mmol) was added pyrrolidine [AH-8] (113 mg, 2 eq., 1.59 mmol) at room temperature. The reaction mixture was heated up to 55°C for 16h, with progress of the reaction being monitored by ELSD/TLC. The reaction mixture was concentrated at 45°C under reduced pressure, and purified by silica gel column chromatography using 2-5% MeOH in DCM to get product as HBr salt. The salt was dissolved in dry dichloromethane (3 ml), potassium carbonate (2.0 eq) added to the solution, and allowed to stir for 90 minutes. The resulting solution was filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to afforded tris(5-(octanoyloxy)pentyl) 2-((6-(pyrrolidin-1-yl)hexanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-009] (174 mg, yield 21.96%) as yellow liquid.

[01008] ^1H NMR (400 MHz, CDCl_3): δ 4.14 (t, J = 6.64 Hz, 2H), 4.08- 4.03 (m, 9H), 3.29 - 3.25 (d, J = 15.52 Hz, 2H), 3.20-3.16 (d, J = 15.52 Hz, 2H), 2.66 (brs, 5H), 2.33-2.26 (m, 8H), 2.92 (brs, 4H), 1.71-1.58 (m, 22H), 1.44 -1.34 (m, 10H), 1.29-1.28 (brs, 24H), 0.87 (t, J = 6.4 Hz, 9H). ELSD analysis: Purity 99.74 %, Calculated $\text{C}_{55}\text{H}_{97}\text{NO}_{14}$ = 995.69, Observed = 996.45 (m/z , $\text{M}+\text{H}^+$). CAD Purity = 93.40 %.

Example 40R. Synthesis of JC-24-TL-010 (Compound 116)

Synthetic Protocol AH

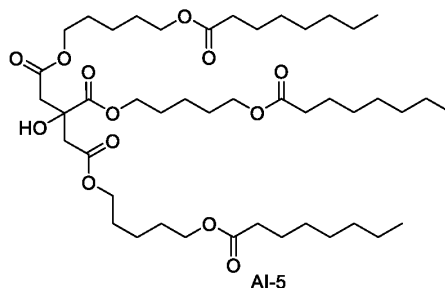
5 **Intermediate [Al-3]:**

- [01009] To a stirred solution of 5-bromo-1-pentanol [Al-2] (25 g, 150 mmol) in dichloromethane (257 mL, 4.02 mol) was added triethylamine (52.2 mL, 2.5 eq., 374 mmol). Reaction mass was cooled to 0 °C and octanoyl chloride [Al-1] (25.5 mL, 150 mmol) was added drop wise. Reaction mass allowed to stir at RT for 16 h, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction mass was diluted with water (500 ml) and extracted with dichloromethane (2x100 ml). Combined organic layer was washed with sodium bicarbonate solution (1000 ml). Organic layer was dried over sodium sulphate, filtered, and concentrated under reduced pressure. The crude was purified over silica get by using flash chromatography and 5-10% ethyl acetate to give 5-bromopentyl octanoate [Al-3] (31 g, yield 71.26 %) as colourless liquid.

[01010] **Note:** Two batches of 25.0 g (total 50 g) were performed yielding total 62 g of desired product.

[01011] ^1H NMR (400 MHz, CDCl_3): 4.07 (t, $J = 6.52$ Hz, 2H), 3.40 (t, $J = 6.72$ Hz, 2H), 2.31-2.27 (t, $J = 2.68$ Hz, 2H), 1.92 - 1.85 (m, 2H), 1.69 - 1.59 (m, 4H), 1.54 - 1.43 (m, 2H), 1.34-1.22 (m, 8H), 0.87 (t, $J = 6.76$ Hz, 3H).

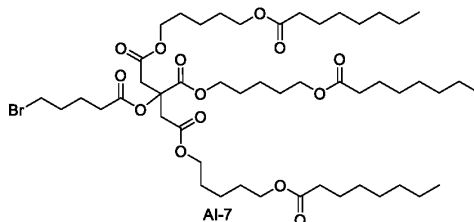
5 **Intermediate [AI-5]:**



[01012] To a stirred solution of 5-bromopentyl octanoate [AI-3] (61.1 g, 3.8 eq., 187 mmol) and 2-hydroxy-1,2,3-propanetricarboxylic acid [AI-4] (9.6 g, 50 mmol) in dimethylformamide (480 mL, 6.2 mol) was added TEA (69.6 mL, 10 eq., 0.5 mol). The reaction mixture was stirred for 16 h at 60 °C, with the progress of reaction being monitored by ELSD/TLC. After consumption of the starting material, the reaction was cooled to RT and then quenched with water (2000 mL) and extracted with diethyl ether (2x 500 mL). The organic layer dried over sodium sulphate, filtered, and concentrated under reduced pressure. Resulting crude was purified over silica using 20% ethyl acetate in n-hexane to obtained tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [AI-5] (21 g, yield-50.69%) as light yellow colour liquid.

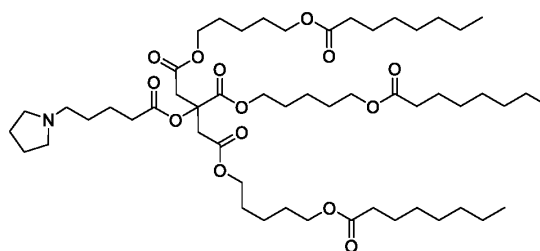
[01013] ^1H NMR (400 MHz, CDCl_3): 4.21 (t, $J = 6.68$ Hz, 2H), 4.12-4.03 (m, 10H), 2.89- 2.85 (d, $J = 15.50$ Hz, 2H), 2.80-2.76 (d, $J = 15.6$ Hz, 2H), 2.30 (t, $J = 6.4$ Hz, 6H), 1.75 -1.56 (m, 18H), 1.45 -1.37 (m, 6H), 1.31-1.26 (m, 22H), 0.87 (t, $J = 9.0$ Hz, 9H).

Intermediate [AI-7]:



[01014] To a stirred solution of 5-bromovaleric acid [AI-6] (1.75 g, 4 eq., 9.65 mmol) in dichloromethane (25 mL, 390 mmol), were added N,N-dimethyl-4-pyridylamine (589 mg, 2 eq., 4.82 mmol), and EDC.HCl (2.31 g, 5 eq., 12.1 mmol) at room temperature and stirred for 10 minute at same temperature. After this time, tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [AI-5] (2 g, 2.41 mmol) was added to the resulting reaction mixture and allowed to stir at room temperature for 48h, with monitoring by TLC/ELSD. After consumption of the starting material, the reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (2x 30 mL). The combined organic layer dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting crude material was purified by using silica gel flash column chromatography and 5-10% ethyl acetate in heptane. The pure fraction were combined and concentrated under reduced pressure to obtained tris(5-(octanoyloxy)pentyl) 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate [AI-7] (1 g, yield 41.78%) as a colourless liquid. ELSD analysis: Purity 98.70 %, Calculated C₅₀H₈₇BrO₁₄ = 990.53, Observed = 993.35 (m/z, M+H⁺).

15 **JC-24-TL-010 (Compound 116):**



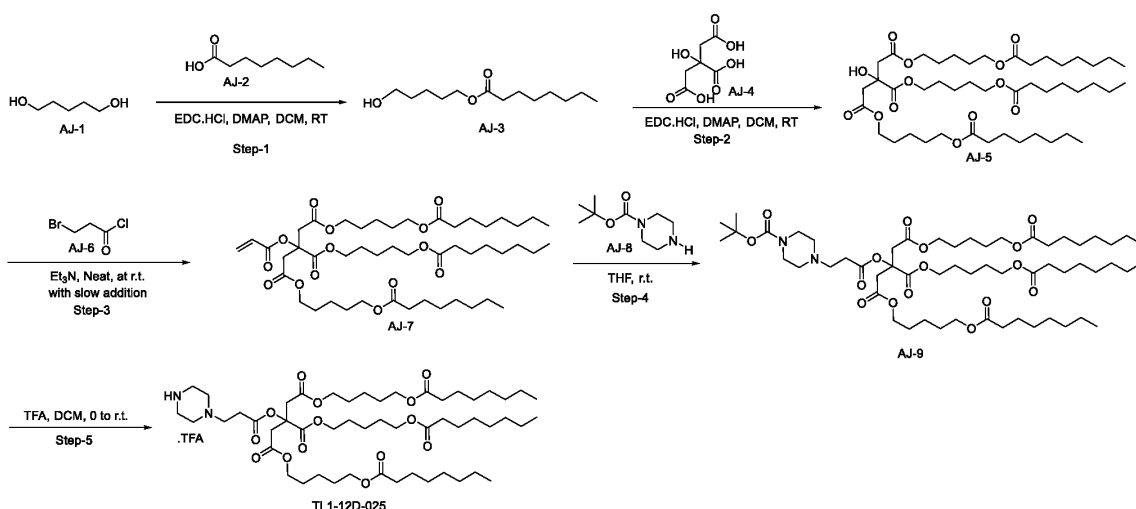
JC-24-TL-010

[01015] To a stirred solution of tris(5-(octanoyloxy)pentyl) 2-((5-bromopentanoyl)oxy)propane-1,2,3-tricarboxylate [AI-7] (1 g, 1.01 mmol) in tetrahydrofuran (8.33 mL, 102 mmol) was added pyrrolidine [AI-8] (179 mg, 2.5 eq., 2.52 mmol) at room temperature. The reaction mixture was heated up to 55°C for 16h, with progress of the reaction being monitored by ELSD/TLC. Reaction mixture was concentrated at 45°C under reduced pressure and purified by silica gel column chromatography (2-5% MeOH in DCM) to get product as HBr salt. The salt was dissolved in dry dichloromethane (3 ml), potassium carbonate (2.0 eq) added to the solution, and allowed to stir for 90 minutes. The resulting solution filtered through 0.2 mm membrane filter. Filtrate was evaporated under reduced pressure to get tris(5-(octanoyloxy)pentyl) 2-((5-(pyrrolidin-1-yl)pentanoyl)oxy)propane-1,2,3-tricarboxylate [JC-24-TL-010] (114 mg, yield -11.51%) as a yellow liquid.

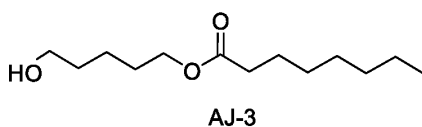
- [01016] ^1H NMR (400 MHz, CDCl_3): δ 4.15 (t, J = 6.6 Hz, 2H), 4.12-4.03 (m, 10H), 3.28-3.24 (d, J = 15.48 Hz, 2H), 3.19-3.11 (d, J = 15.48 Hz, 2H), 2.84 (brs, 4H), 2.36 (t, J = 7.16 Hz, 2H), 2.28 (t, J = 7.56 Hz, 6H), 2.01 (brs, 4H), 1.85-1.83 (m, 2H), 1.71-1.58 (m, 22H), 1.44-1.36 (m, 6H), 1.29-1.27 (m, 24H), 0.87 (t, J = 7.0 Hz, 9H). ELSD analysis: Purity 99.77 %, Calculated $\text{C}_{54}\text{H}_{95}\text{NO}_{14}$ = 981.68, Observed = 982.45 (m/z , $\text{M}+\text{H}^+$). CAD Purity = 92.00 %.

Example 40S. Synthesis of TL1-12D-025 (Compound 126)

Synthetic Protocol AI



Intermediate [AJ-3]:



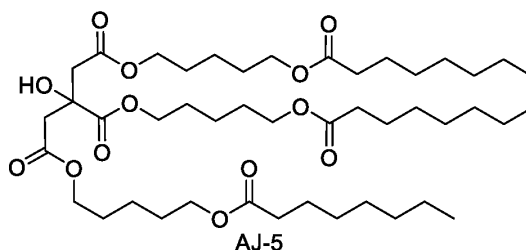
10

- [01017] To a stirred solution of octanoic acid [AJ-2] (100.0 g, 693 mmol) and pentane-1,5-diol [AJ-1] (65.0 g, 624 mmol) in dichloromethane (2000 mL) was cooled to 0 °C and ({[3-(dimethylamino)propyl]imino}methyldene)(ethyl)amine hydrochloride (167.0 g, 874 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (17.1 g, 139 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored by ELSD/TLC (SM was consumed). Water (200 mL) was added to the reaction mixture, and extract with DCM (3x500 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-10% Ethyl acetate in Hexane), to give the desired 5-hydroxypentyl octanoate [AJ-3] (60.0 g, yield 37.56%) as a yellow liquid.

15

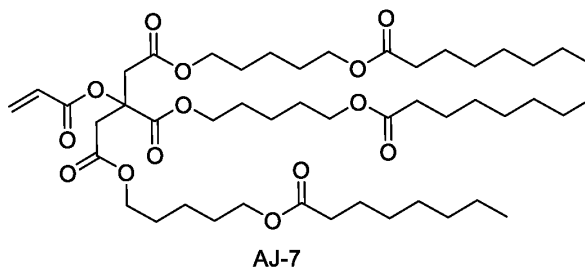
[01018] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.10-4.07 (m, 2H), 3.67 (bs, 2H), 2.32-2.28 (m, 2H), 1.71-1.58 (m, 5H), 1.48-1.42 (m, 2H), 1.36-1.29 (m, 9H), 0.90-0.87 (m, 3H).

Intermediate [AJ-5]:



- 5 [01019] To a stirred solution of 2-hydroxypropane-1,2,3-tricarboxylic acid [AJ-4] (12.5 g, 65.1 mmol) and 5-hydroxypentyl octanoate [AJ-3] (60.0 g, 260 mmol) in dichloromethane (500 mL) was cooled to 0 °C and ([3-(dimethylamino)propyl]imino)methylidene(ethyl)amine hydrochloride (49.9 g, 260 mmol) was added followed by 4-(dimethylamino)pyridin-1-ium (8.02 g, 65.1 mmol). The reaction mixture stirred at r.t. for 48 h. The progress of reaction was monitored
- 10 by ELSD/TLC (SM was consumed). Water (50 mL) was added to the reaction mixture, and extract with DCM (3x100 mL). The resulting organic layer was dried over Na_2SO_4 , and concentrated under reduce pressure, and the crude was purified by flash column chromatography (SiO_2 : 0-30% Ethyl acetate in Hexanes), to give the desired tris(5-(octanoyloxy)pentyl) 2-hydroxypropane-1,2,3-tricarboxylate [AJ-5] (21.0 g, yield 38.9%) as yellow liquid.
- 15 [01020] $^1\text{H-NMR}$ (400 MHz, CDCl_3)- δ 4.23-4.20 (m, 2H), 4.10-4.04 (m, 10H), 2.89-2.77 (dd, J = 15.6, 19.2 Hz, 4H), 2.30-2.26 (m, 6H), 1.75-1.56 (m, 18H), 1.45-1.36 (m, 6H), 1.29-1.28 (m, 24H), 0.88 (m, 9H). *ELSD analysis*: Purity 97.90 %, Calculated $\text{C}_{45}\text{H}_{80}\text{O}_{13}$ = 828.56, Observed = 829.35 (m/z , $\text{M}+\text{H}^+$).

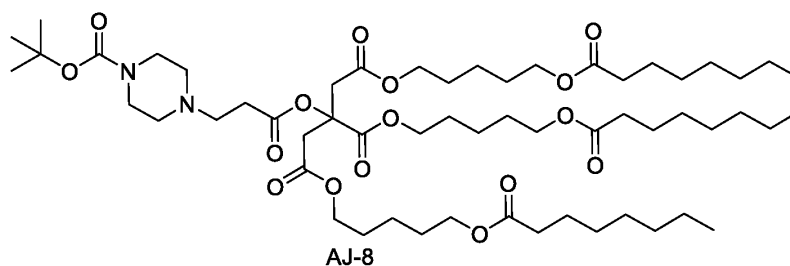
Intermediate [AJ-7]:



[01021] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-hydroxypropane-1,2,3-tricarboxylate [AJ-5] (10 g, 12.1 mmol) in triethylamine (6.10 g, 60.3 mmol) was added 3-bromopropanoyl chloride (1.21 mL, 12.1 mmol) was successively at 0 °C and stirred for 16h at same 25 °C temperature. TLC shows SM consumed and formed new spot. The reaction mass was filtered through celite and washed with ethyl acetate to get crude product. The crude was purified by flash column chromatography (SiO₂: 0-30% Ethyl acetate in Hexanes), to give the desired [AJ-7] (2.0 g, yield 18.7%) as yellow liquid.

[01022] ¹H NMR (400 MHz, CDCl₃): δ 6.42 (d, *J* = 17.2 Hz, 1H), 6.12-6.05 (m, 1H), 5.89 (d, *J* = 10.4 Hz, 1H), 4.17 (t, *J* = 6.4 Hz, 2 H), 4.09-4.03 (m, 10H), 3.34 (d, *J* = 15.2 Hz, 2H), 3.25 (d, *J* = 15.6 Hz, 2H), 2.28 (t, *J* = 7.2 Hz, 6 H), 1.70-1.59 (m, 18H), 1.43-1.35 (m, 6H), 1.28 (s, 24H), 0.87 (t, *J* = 6.8 Hz, 9 H). *ELSD analysis*: Purity 99.84%, Calculated C₄₈H₈₂O₁₄ = 882.57, Observed = 883.40 (*m/z*, M+H⁺).

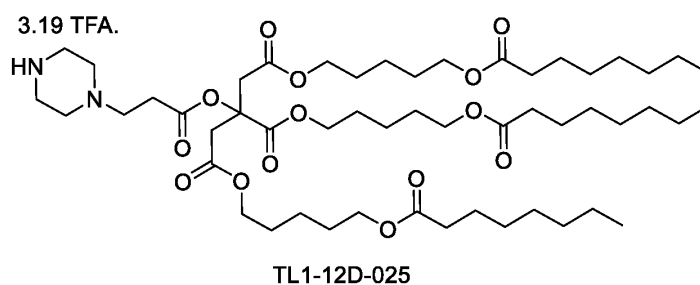
Intermediate [AJ-8]:



[01023] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-(prop-2-enoyloxy)propane-1,2,3-tricarboxylate [AJ-7] (0.5 g, 566 μmol) in tetrahydrofuran (5 mL, 61.4 mmol), added 4-[(tert-butoxy)carbonyl]piperazin-1-ium [AJ-8] (117 mg, 623 μmol). Reaction mass was stirred at RT for 48 h. Progress of the reaction was monitored by TLC. Reaction mass was distilled out under reduced pressure and resulting crude material was purified by flash column chromatography (SiO₂: 0-100% Ethyl acetate in Hexanes), to give 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-{4-[(tert-butoxy)carbonyl]piperazin-1-yl}propanoyl)oxy]propane-1,2,3-tricarboxylate [AJ-9] (0.5 g, yield 88%) as a yellow liquid.

[01024] *ELSD analysis*: Purity 95.97 %, Calculated C₅₇H₁₀₀N₂O₁₆ = 1068.71, Observed = 1069.75 (*m/z*, M+H⁺).

TL1-12D-025 (Compound 126):



[01025] To a stirred solution of 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-{4-[(tert-butoxy)carbonyl]piperazin-1-yl}propanoyl)oxy]propane-1,2,3-tricarboxylate [AJ-9] (0.5 g, 468 μmol) in dichloromethane (5 mL, 78.1 mmol), trifluoroacetic acid (179 μL , 2.34 mmol) was added at 0 °C. The resulting reaction mixture was stirred for 6 h at room temperature. The progress of reaction mass was monitored by ELSD/TLC (SM was consumed). Reaction mass was lyophilized and obtained 1,2,3-tris[5-(octanoyloxy)pentyl] 2-[(3-(piperazin-1-yl)propanoyl)oxy]propane-1,2,3-tricarboxylate [TL1-12D-025] (325 mg, yield 71.71%) as a Yellow liquid.

[01026] ^1H NMR (400 MHz, CDCl_3): δ 4.20 (t, J = 6.8 Hz, 2H), 4.09 (m, 10H), 3.69 (s, 8H), 3.48-3.47 (m, 2H), 3.22 (s, 4H), 2.91-2.88 (m, 2H), 2.28 (t, J = 7.6 Hz, 6 H), 1.71-1.56 (m, 18H), 1.43-1.37 (m, 6 H), 1.31-1.28 (m, 24H), 0.87 (t, J = 6.8 Hz, 9H). *ELSD analysis*: Purity 99.64%, Calculated $\text{C}_{52}\text{H}_{92}\text{N}_2\text{O}_{14}$ = 968.65, Observed = 969.65 (m/z , $\text{M}+\text{H}^+$).

Example 41. Lipid nanoparticle formulation

[01027] Ionizable lipids described herein can be used in the preparation of lipid nanoparticles according to methods known in the art. For example, suitable methods include methods described in International Publication No. WO 2018/089801, which is hereby incorporated by reference in its entirety.

[01028] The lipid nanoparticles in the examples of the present invention were formulated using *Process A* of WO 2018/089801 (see, e.g., Example 1 and Figure 1 of WO 2018/089801). *Process A* ("A") relates to a method of encapsulating mRNA by mixing mRNA with a mixture of lipids, without first pre-forming the lipids into lipid nanoparticles. In an exemplary process, an ethanolic solution of a mixture of lipids (cationic lipid, phosphatidylethanolamine, cholesterol, and polyethylene glycol-lipid) at a fixed lipid to mRNA ratio were combined with an aqueous buffered solution of target mRNA at an acidic pH under controlled conditions to yield a suspension of uniform LNPs. After ultrafiltration and diafiltration into a suitable diluent system, the resulting nanoparticle suspensions were diluted to final concentration, filtered, and stored frozen at -80°C until use.

Compound No.	6h			24h		
	Mean (ng/mL)	SD	N	Mean (ng/mL)	SD	N
36	15.2	4.1	4	11.8	4.6	4
35	14.4	5.7	4	8.2	3	4
34	12.4	8	4	7.7	4.5	4
33	11.5	2.2	4	9.2	2.6	4
76	11.36	1.9	4	8.4	0.6	4
32	10.1	3.4	4	7.4	3.2	4

Compound No.	6h			24h		
	Mean (ng/mL)	SD	N	Mean (ng/mL)	SD	N
31	9.34	2.62	4	10.91	3.91	4
59	9.3	5.3	4	4.4	2.7	4
30	9	2.7	4	5.1	1	4
29	8.9	1.8	4	6.5	0.7	4
58	8.5	0.7	4	5.1	1	4
57	7.9	2.2	4	5.1	1.7	4
56	7.1	4.1	4	5.2	2.9	4
75	6.1	2.2	4	3.5	1.3	4
55	5.7	2.2	4	3.4	1	4
28	5.5	2.5	4	4.2	1.5	4
27	5.4	0.9	4	2.3	0.2	4
54	5.2	2.4	4	3.3	1.1	4
74	5.17	2.4	4	3.3	1.2	4
26	4.8	1.8	4	2.5	1	4
25	4.2	0.9	4	2.2	0.4	4
D-Lin- MC3-DMA	3.94	1.24	64	2.44	0.63	64
53	3.5	1.4	4	2.1	1.1	4
52	3.18	0.61	4	2.38	0.3	4
24	3	0.6	4	1	0.4	4
22	2	0.6	4	1.1	0.6	4
21	1.65	0.67	4	0.86	0.45	4
47	1.14	0.75	4	0.63	0.3	4
73	1.08	0.52	4	0.68	0.24	4
16	0.93	0.31	4	0.59	0.28	4
71	0.83	0	4	0.83	0.01	4
12	0.8	0	4	0.8	0	4
8	0.8	0	4	0.8	0	4
11	0.8	0	4	0.8	0	4
6	0.5	0.2	4	0.2	0.2	4

Compound No.	6h			24h		
	Mean (ng/mL)	SD	N	Mean (ng/mL)	SD	N
4	0.22	0.07	4	0.2	0.05	4
1	0.17	0.02	4	0.08	0.03	4
41	0.1	0.07	4	0.14	0.02	4
65	0.1	0.1	4	0.1	0.1	4

Example 43. Delivery of Firefly Luciferase (FFL) mRNA by intranasal administration

[01031] Lipid nanoparticle formulation 4 listed in Table 4 comprising FFL mRNA, cationic lipid, DMG-PEG2000, cholesterol and DOPE was administered in mice via pipetting the formulations at 10µg/Animal and 15µl per nostril. On Day 2, 24 hours post dose (±5%), all animals underwent a luminescent imaging session using IVIS with separate ROIs on the nose and lungs. Whole body imaging was performed 10-15 minutes following D-Luciferin administration. All animals were dosed with 0.2 mL of 15 mg/mL D-luciferin solution via intraperitoneal (IP) injection. Anesthesia was performed by isoflurane during the procedure and animals were placed sternal recumbency (face-down). The intranasal vaccine drug product may be administered via nasal spray. Exemplary data are provided in Table 6, which describes the average radiance in p/s/cm²/sr (the number of photons per second that leave a square centimeter of tissue and radiate into a solid angle of one steradian (sr))

Table 6. Exemplary in vivo protein expression following intranasal administration

Compound No.	Mean radiance (p/s/cm²/sr)	SD	N
64	1669900	715189	5
33	430740	176776	5
29	383780	80000	5
69	340240	100000	5
32	332460	100000	5
54	298936	215000	5
57	297500	90000	5
34	265080	262000	5
56	257846	233000	5
63	249446	186946.2	5
76	233720	100000	5

Compound No.	Mean radiance (p/s/cm ² /sr)	SD	N
74	215407	240000	5
35	182972	80000	5
28	165870	90000	5
53	160170	209000	5
48	142660	37200	5
6	135582	54400	5
41	134710	135000	5
75	130552	50000	5
25	115972	55500	5
30	114168	20600	5
26	93064	29300	5
50	78372	40000	5
12	76242	30000	5
89	74148	43381	5
31	39496	26600	5
39	23571	12278	5
4	18200	11800	5
68	8334	5030	5
21	6040	6060	5
61	1858	393	5
13	1762	300	5

Example 44. Delivery of Firefly Luciferase (FFL) mRNA by pulmonary administration

[01032] Lipid nanoparticle formulation 4 listed in Table 4 comprising FFL mRNA, cationic lipid, DMG-PEG2000, cholesterol and DOPE was administered to male CD1 mice (6-8 weeks old) by a single intratracheal administration via Catheter (50µl/animal) while under anesthesia. At approximately 24 hours post-dose, the animals were dosed with luciferin at 150 mg/kg (60 mg/ml) by intraperitoneal injection at 2.5ml/kg. After 5-15 minutes, all animals were imaged using an IVIS imaging system to measure luciferase production in the lung. Table 7 provides the the average radiance in p/s/cm²/sr and shows that lipid nanoparticles comprising the cationic lipids described herein are effective in delivering FFL mRNA *in vivo* based on positive luciferase activity.

Table 7. Exemplary in vivo protein expression following pulmonary administration

Compound Number	Mean radiance (p/s/cm ² /sr)	SD	N
74	2563400	1854431	5
76	1842000	904857.2	5
21	1235560	608837.8	5
65	855600	440917.6	5
88	804000	373341	5
47	768260	417044	5
49	578147.5	600937.8	4
52	564775	203896	4
26	556900	223542.1	4
87	512706	523300.9	5
86	462000	303432.9	5
17	417460	236573.4	5
85	370086	372436.5	5
2	292300	134871.3	5
4	291340	94604.67	5
72	286557.4	273995.3	5
84	251800	117024.4	5
68	232800	286402.1	5
41	180110	155184	3
31	91460	22118.3	2
69	88282	79168.39	5
1	60513.6	81775.25	5
3	18390.4	7872.576	5
60	3389.2	852.4592	5
77	1718	627.511	5
16	1691.6	440.9068	5

Example 45. Intranasal dosing in a mouse influenza virus challenge model

[01033] To test the efficacy of intranasal dosing in a mouse influenza virus challenge model,

- 5 BALB/c mice were inoculated intranasally with 10 ug of Lipid Nanoparticles (LNPs) encapsulating

CA09 HA mRNA (these LNPs comprised either Compound 64 or the lipid OF-02 [OF-02 is published in Fenton et al., 2016, Adv. Mater., 28:2939-43]) or intramuscularly with 1 ug of LNPs encapsulating CA09 HA mRNA (these LNPs comprised OF-02 only) at days 0 and 21, along with a negative control group with two doses of phosphate-buffered saline (PBS) buffer administered intranasally. At day 35, all mice were challenged intranasally with A/California/07/09 (H1N1), at 2.5 E+5 TCID50. All mice administered LNPs comprising lipid Compound 64 intranasally or LNPs comprising OF-02 intramuscularly had no mortality 14 days post-challenge (see Figures 1 and 2). However, those in the PBS control group suffered significant and rapid weight loss which led to 100% mortality by day 6 (see Figures 1 and 2). Mice administered LNPs comprising OF-02 intranasally had some protection against mortality, with 43% surviving at day 14 post-challenge. These results demonstrated high efficacy of LNPs comprising Compound 64 administered intranasally in a lethal mouse influenza challenge model.

Table 8: Lipid Nanoparticle (LNP) formulation details for example 45

Ionizable lipid	LNP formulation
Compound 64	3% DMG-PEG-2k : 40% Compound 64 : 27% Chol : 30% DOPE
OF-02	1.5% DMG-PEG-2k : 40% OF-02 : 28.5% Chol : 30% DOPE

Example 46. Delivery of Firefly Luciferase (FFL) mRNA by intranasal administration
Lipid nanoparticle formulation 3 listed in Table 4 comprising FFL mRNA, cationic lipid, DMG-PEG2000, cholesterol and DOPE was administered in mice via pipetting the formulations at 10µg/Animal and 15µl per nostril. On Day 2, 24 hours post dose (±5%), all animals underwent a luminescent imaging session using IVIS. Whole body imaging was performed 10-15 minutes following D-Luciferin administration. All animals were dosed with 0.2 mL of 15 mg/mL D-luciferin solution via intraperitoneal (IP) injection. Anesthesia was performed by isoflurane during the procedure and animals were placed 10 sternal recumbency (face-down). Exemplary data are provided in Table 9, which describes the average radiance in p/s/cm²/sr (the number of photons per second that leave a square centimeter of tissue and radiate into a solid angle of one steradian (sr)).

Table 9. Exemplary in vivo protein expression following intranasal administration

Compound No.	Mean radiance (p/s/cm²/sr)	SD	N
64	80600	48313	5
112	72686	54088	5

113	78020	28286	5
114	83878	73360	5
115	119604	47171	5
116	133262	61929	5
118	57088	24354	5
119	107106	50172	5
120	82266	34723	5
121	58050	22715	5
122	28438	5626	5

Example 47. Comparison of intranasal administration of Compound 64 with intramuscular administration of OF-02

- 5 To test the immune responses of mice to intranasal dosing, BALB/c mice were inoculated intranasally with 10 ug of Compound 64 LNPs encapsulating CA09 HA mRNA or intramuscularly with 1 ug of OF-02 LNPs encapsulating CA09 HA mRNA at days 0 and 21, along with a negative control group with 10% trehalose buffer administered intranasally.

10 **Distant mucosal activation (IgG/IgA ELISA in vaginal washes)**

ELISA Assays

Immunoglobulin A (IgA) ELISA

- 15 For the IgA ELISA, Influenza H1 A/Cal/09 protein was captured on 96 well polystyrene plates (Nunc MaxiSorp 439454) at a concentration of 1 µg/mL in carbonate-bicarbonate buffer. The plates were coated with 50 µL/well of antigen (1 µg/mL) and incubated overnight (16 ± 4 hours) at 2-8°C. After overnight incubation, the antigen coated plates were washed 5 times with washing buffer (PBS, 0.05% Tween20), and blocked with blocking solution (PBS, 10% BSA). The plates were incubated
- 20 for 30 minutes at room temperature. Test samples, naïve control, and reference samples (if available) were diluted (serum - 1:10 or 1:20; BAL, nasal wash, vaginal wash - 1:5) followed by a 4-fold serial dilution in sample diluent (PBS, 1% BSA, 0.05% Tween 20) and added to wells in duplicates, followed by incubation at room temperature for 60 minutes. Plates were washed 5 times with washing buffer, and goat anti-mouse HRP IgA (Bethyl A90-103P) was added at a dilution

of 1:10,000. Plates were incubated 30 minutes at room temperature, then washed 5 times with washing buffer. TMB substrate (Sera Care 5120-0075) was added to each plate, and the reaction was stopped after 15 minutes of incubation at room temperature away from light, with TMB stop solution (Sera Care 5120-0020). Plates were read at 450 nm with a Thermo Labsystems Multiskan spectrophotometer. The anti-HA specific antibody titers are expressed as a reciprocal of the highest serum dilution with an absorbance value >0.3.

Immunoglobulin G (IgG) ELISA

For the IgG ELISA, Influenza H1 A/Cal/09 protein (Provided by Sanofi) was captured on 96 well polystyrene plates (Nunc MaxiSorp 439454) at a concentration of 1 µg/mL in carbonate-bicarbonate buffer. The plates were coated with 50 µL/well of antigen and incubated overnight (16 ± 4 hours) at 2-8°C. After overnight incubation, the antigen coated plates were washed 3 times with washing buffer (PBS 0.05% Tween20) and blocked with blocking solution (1.0% BSA in PBS). Test samples, naïve control and reference sample were diluted (serum - 1:10 or 1:20; BAL, nasal wash, vaginal wash - 1:5) followed by a 1:5 dilution in sample diluent (PBS 1% BSA 0.05% Tween 20) and added to wells in duplicates, followed by incubation at room temperature for 120 minutes. Anti-mouse HRP IgG (Invitrogen 314300) was added at a dilution of 1:10,000, followed by incubation at room temperature for 60 minutes. Plates were washed 5 times with washing buffer. Excess HRP IgG was washed with PBS. TrueBlue substrate (SERA CARE 10344659) was added to each plate and the reaction was stopped after 10 minutes of incubation away from light, with HCL. Plates were read at 450 nm with a Thermo Labsystems Multiskan spectrophotometer. The anti-HA specific antibody titers are expressed as a reciprocal of the highest serum dilution with an absorbance value >0.3.

Lung CD4 memory T cells

Lung Tissue Processing for Flow Cytometry Analysis:

Lung tissue was transferred to a 6-well dish and mince using a sterile scalpel. The minced lung tissue was transferred to a labelled 50 mL conical tube. The 6-well dish was washed with 5 mL of Digestion Buffer (5 mg/mL Collagenase, Type 1 + 0.2 mg/mL DNase I in BSA Buffer (PBS + 0.5% BSA)) and added to 50 mL tube containing tissue sample. The tissue sample was incubated at 37°C for 30 minutes in an orbital shaker at 150 rpm. The reaction was stopped with 10 mL of cold BSA Buffer and the sample filtered into a new 50 mL conical tuber using a 70 µm strainer. The tissue was

pressed through a strainer using the plunger end of a syringe. The strainer was washed with 5 mL of cold BSA Buffer and the cells centrifuged at 400 x g for 10 minutes at 4°C; the supernatant was discarded. The red blood cells were lysed with ACK Lysis Buffer for 1.5 minutes on ice. The cells were washed with cold BSA Buffer and centrifuged at 400 x g for 10 minutes at 4°C; the supernatant was discarded. The cell pellet was resuspended in 1 mL of FBS Buffer (PBS + 5% FBS). The cell count was enumerated with AOPI staining solution using a Nexcelom Cellometer. The cell count for each sample was recorded. Samples were stored in a 37°C incubator until ready for flow cytometry staining.

10 Splenic T cell activation

Spleen Processing for Flow Cytometry Analysis:

Spleen was transferred to a 6-well dish and injected with 1 mL of Digestion Buffer (0.1% (w/v) Collagenase, Type IV + 20 µg/mL DNase I in HBSS containing 1% FBS) using a 23-gauge needle. The tissue was transferred to a labelled 15 mL conical tube containing 4 mL of Digestion Buffer. The tissue was digested for 25 minutes at 37°C. The tissue was minced with scissors and incubated for 10 minutes at 37°C. After incubation, 1 mM/mL of EDTA was added to each tube to stop the enzymatic reaction. The tissue plus solution was transferred to a 100 µm strainer affixed to a labelled 50 mL conical tube. With the plunger end of a syringe, the tissue was pressed through the strainer. The strainer was washed with 10 mL of 1x PBS.

The cells were centrifuged at 1200 rpm for 10 minutes at 4°C; the supernatant was discarded. The red blood cells were lysed with ACK Lysis Buffer for 5 minutes on ice. The cell suspension was washed with cold 1x PBS and transferred to a 100 µm strainer affixed to a new, labelled 15 mL conical tube. The cells were centrifuged at 1200 rpm for 10 minutes at 4°C; the supernatant was discarded. The cell pellet was resuspended in 3 mL of FBS Buffer. The cell count was enumerated with AOPI staining solution using a Nexcelom Cellometer. The cell count for each sample was recorded. Samples were stored in a 37°C incubator until ready for flow cytometry staining.

Flow Cytometry Staining

Samples were stained with LIVE/DEAD Near IR in PBS, followed by Fc-receptor blockade with anti-CD16/CD32 antibody in FBS Stain Buffer for 10 minutes at 4°C, and stained for 30 minutes on ice with antibody cocktail in Brilliant Stain Buffer (BD Biosciences). Excess antibodies were washed

away with FBS Stain Buffer and cells fixed with 2-4% paraformaldehyde. Following fixation, cells are resuspended in 200 μ L of FBS Stain Buffer and stored at 4°C.

Conclusions

- 5 As demonstrated in Fig 3, in these studies it was discovered that intranasal administration of Compound 64 induced lower serum IgG and serum influenza hemagglutination (HAI) titer values than intramuscular administration of OF-02. However, intranasal administration of Compound 64 was found to induce higher IgA in the lung (Fig. 3) and also in a distant mucosal site (vagina) as well as a higher IgG at the distant mucosal site (see Fig. 3). Furthermore, intranasal administration
- 10 of Compound 64 induced greater splenic T cell activation and production of CD4 memory T cells in the lung (Fig.4).

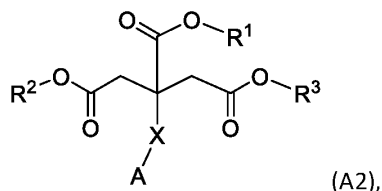
- [01034] From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can
- 15 make various changes and modifications of the invention to adapt it to various usages and conditions.

[01035] All references, patents or applications, U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein in their entireties. Where any inconsistencies arise, material literally disclosed herein controls.

NUMBERED EMBODIMENTS

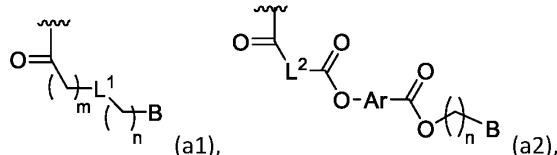
1. A compound having a structure according to

(a) Formula (A2):



- 5 or a pharmaceutically acceptable salt thereof, wherein
 X is independently O or NH;
 each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, C_4 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

A is a substructure selected from:



- 10 $\text{R}^4\text{-B}$ (a3), or $\text{R}^4\text{-B}$ (a4),

B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

- 15 L^1 is a carbonyl, ester, or amide;
 L^2 is C_2 - C_{10} alkylene or a C_2 - C_{10} alkenylene;

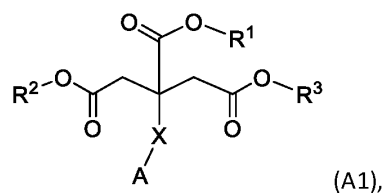
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH_3 , and CH_3 ;

R^4 is C_1 - C_{10} alkylene;

- 20 and wherein A is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently a C_4 - C_{30}

heteroalkyl comprising a disulfide bond or having a structure that is $\text{L}^3\text{-R}^5$, wherein L^3 is OC(O) , CO_2 , or $(\text{O})\text{CO}$; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or L^3 is OC(O) , CO_2 , or $(\text{O})\text{CO}$; o is an integer of 6-12; and R^5 is C_1 - C_6 alkyl;

(b) Formula (A1):

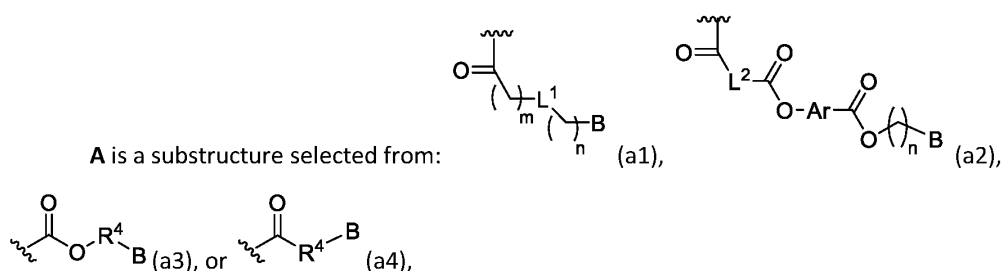


or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

- 5 each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged

- 10 nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

- 15 **Ar** is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH₃, and CH₃;

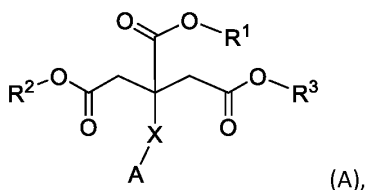
R⁴ is C₁-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R**¹, **R**², and **R**³ is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is ,

- 20 wherein **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R**⁵ is C₄-C₂₄ alkyl; or **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R**⁵ is C₁-C₅ alkyl; or

(c) Formula (A):

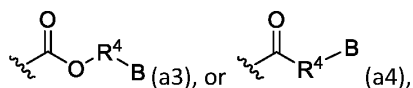
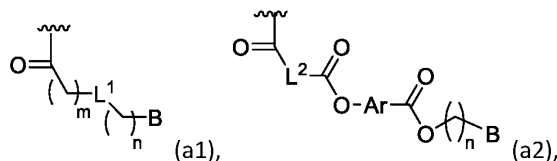


or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀

5 heteroalkyl;



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

10 **m** is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

Ar is phenylene optionally comprising 1-4 substituents independently selected from

15 halogen, OCH₃, and CH₃;

R⁴ is C₂-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R**¹, **R**², and **R**³ is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is $\text{---}(\text{---})_{\text{o}}\text{---L}^3\text{---R}^5$, wherein **L**³ is

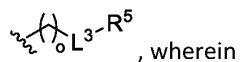
OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R**⁵ is C₄-C₂₄ alkyl; or **L**³ is OC(O), CO₂, or (O)CO; **o** is

20 an integer of 6-12; and **R**⁵ is C₁-C₅ alkyl.

2. The compound of numbered embodiment 1, wherein **X** is O and/or **B** is independently an ionizable nitrogen-containing group.

3. The compound of numbered embodiment 1 or 2, wherein each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, or C₄-C₃₀ alkynyl.

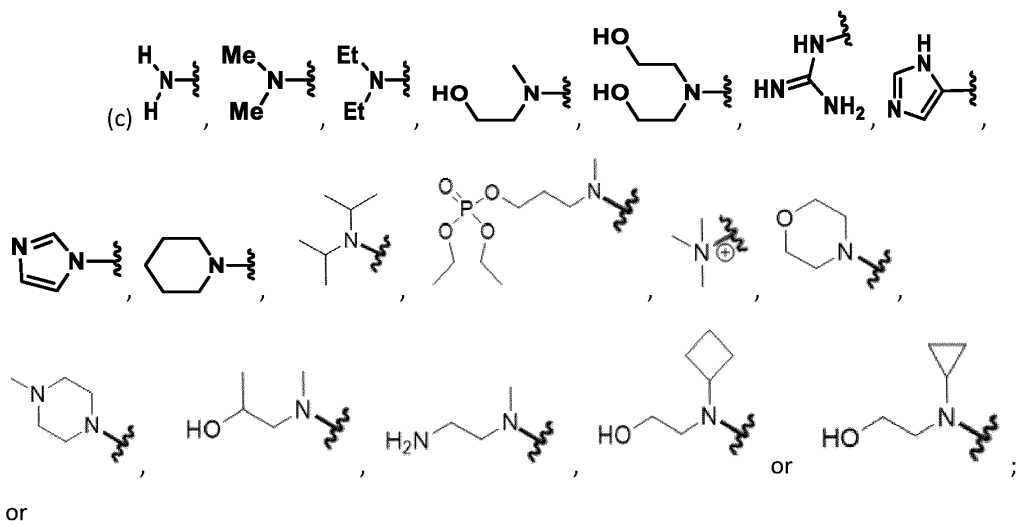
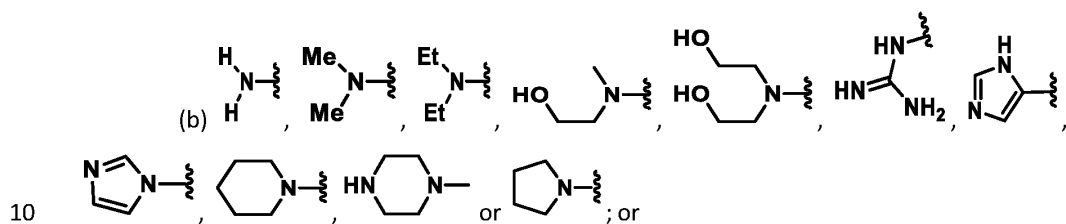
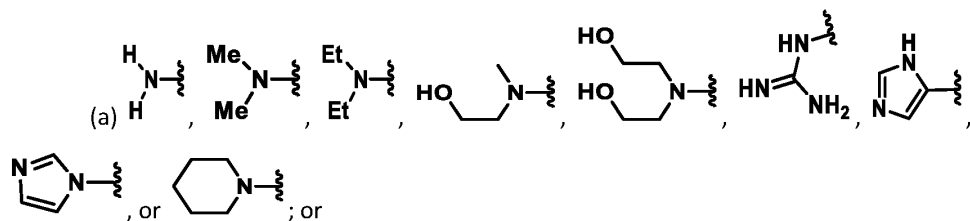
4. The compound of numbered embodiment 1 or 2, wherein each of R^1 , R^2 , and R^3 is independently a C_4 - C_{30} heteroalkyl comprising a disulfide bond or having a structure that is

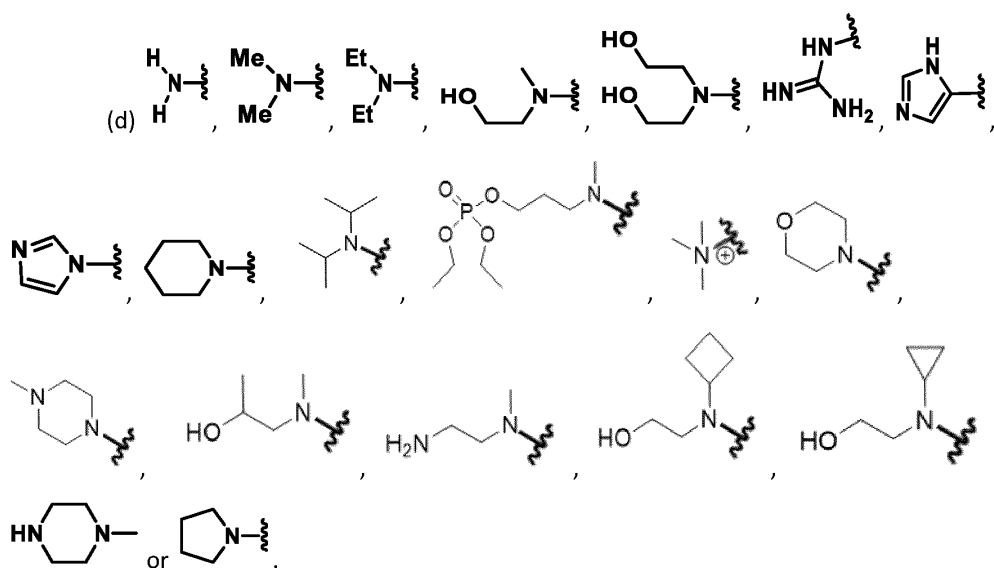


L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl; or

5 L^3 is $OC(O)$, CO_2 , or $(O)CO$; o is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

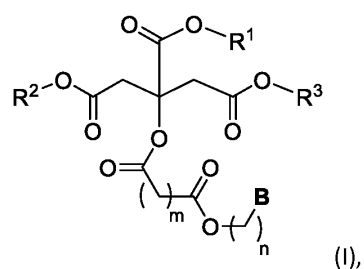
5. The compound of any one of numbered embodiments 1-4, wherein **B** is independently





5

6. The compound of numbered embodiment 1, having a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof, wherein

10 each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

m is an integer of 2-10;

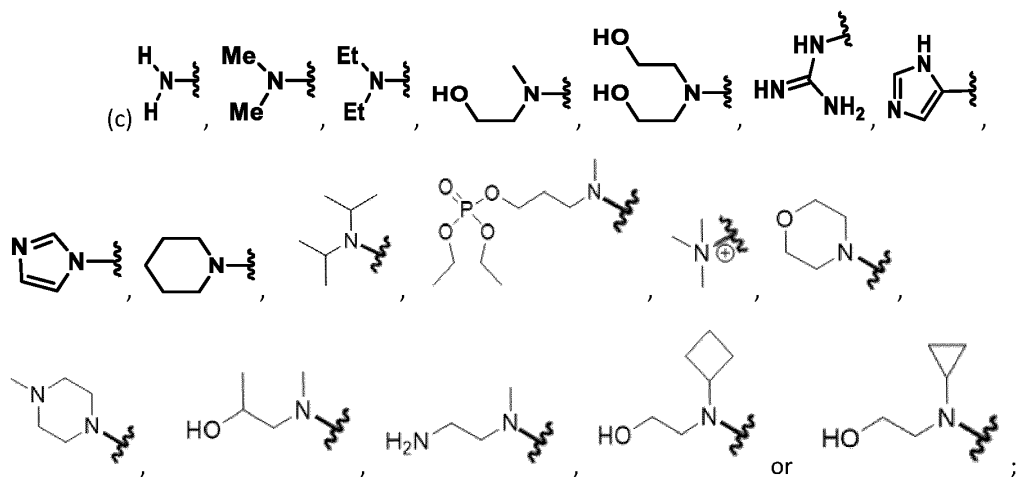
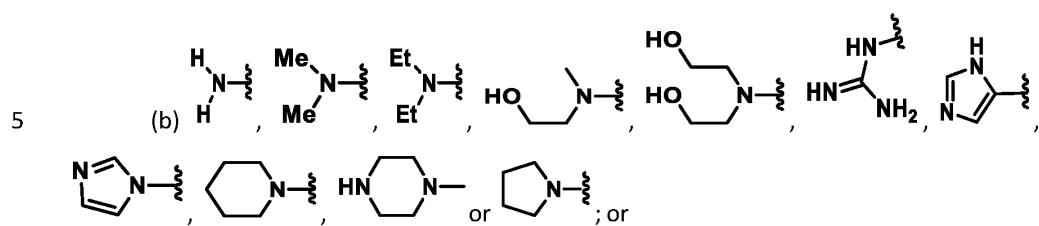
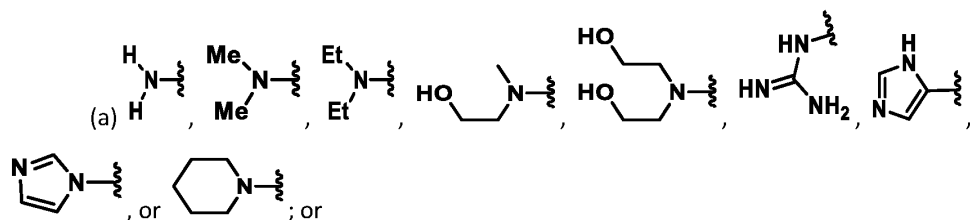
n is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group or a permanently charged
15 nitrogen group.

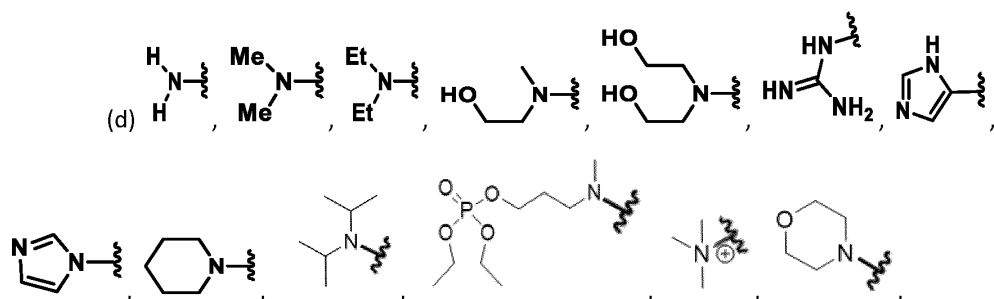
7. The compound of numbered embodiment 6, wherein **B** is independently an ionizable nitrogen containing group.

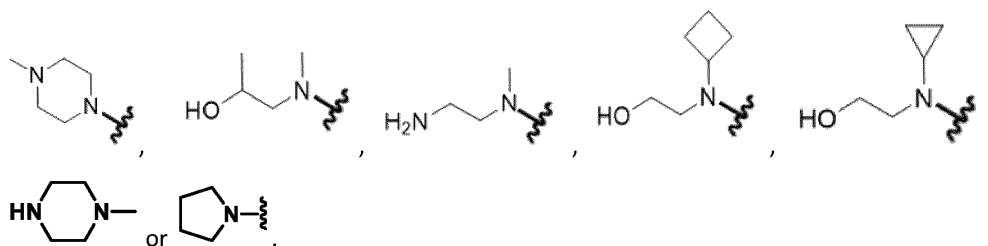
8. The compound of numbered embodiment 6 or 7, wherein m is 2 and/or n is 2 or 3.

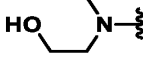
9. The compound of any one of numbered embodiments 6-8, wherein B is independently



10 or

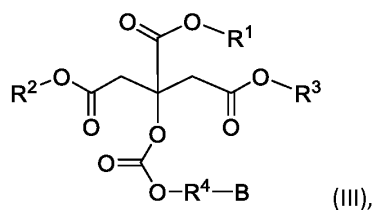




10. The compound of numbered embodiment 9, wherein **B** is  or .

5 11. The compound of numbered embodiment 6, wherein said compound is selected from the group consisting of: Compounds (1), (2), (3), (4), (6), (9), (17), (24), (26), (27), (33), and (35).

12. The compound of numbered embodiment 1, having a structure according to Formula (III):



or a pharmaceutically acceptable salt thereof, wherein

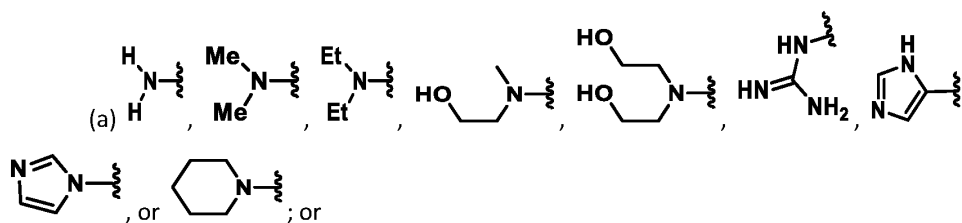
10 each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

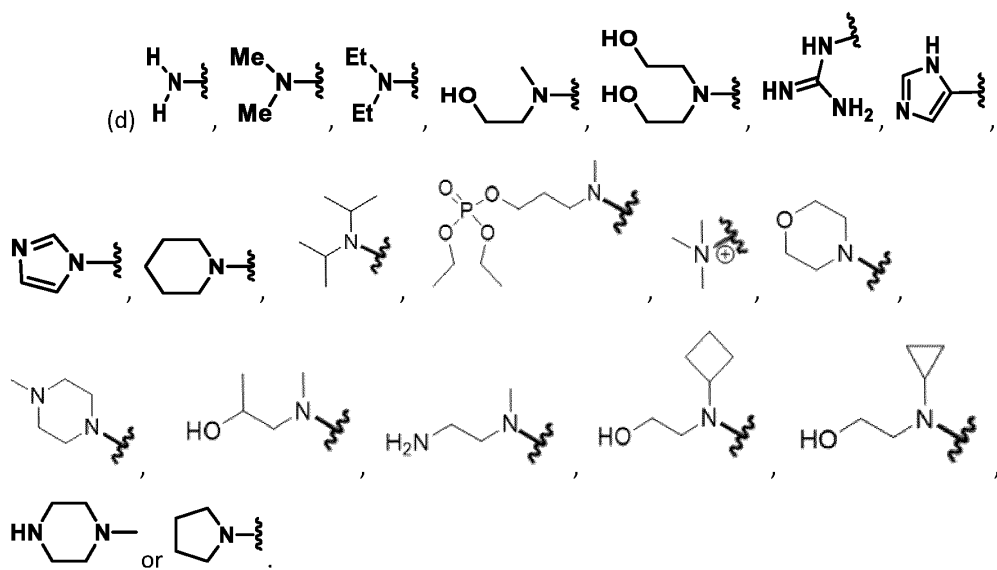
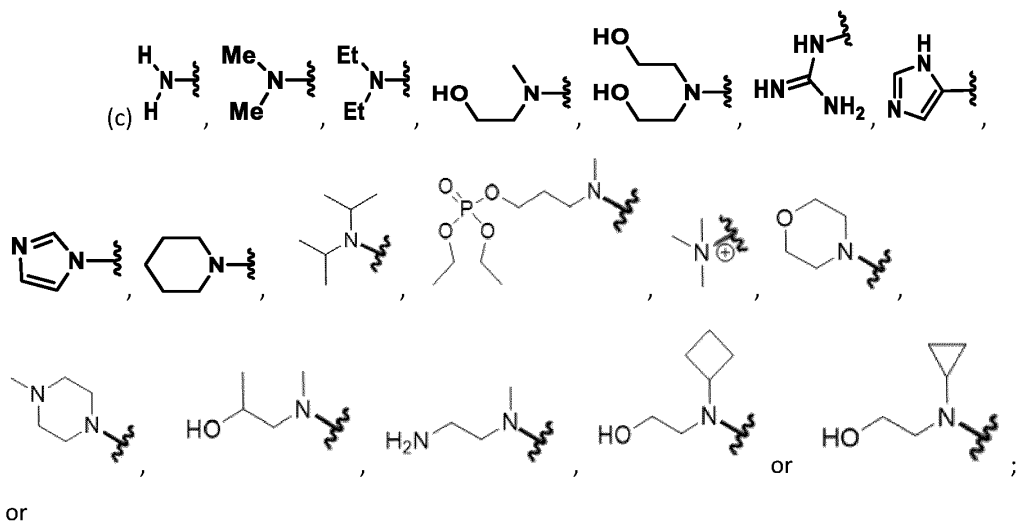
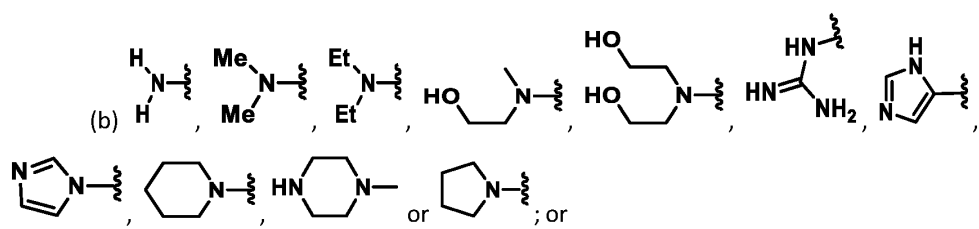
R⁴ is C₂-C₁₀ alkylene; and

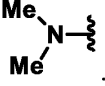
B is independently an ionizable nitrogen-containing group.

13. The compound of numbered embodiment 12, wherein **R**⁴ is -CH₂CH₂-.

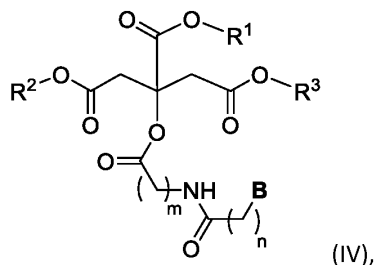
15 14. The compound of numbered embodiment 12 or 13, wherein **B** is independently





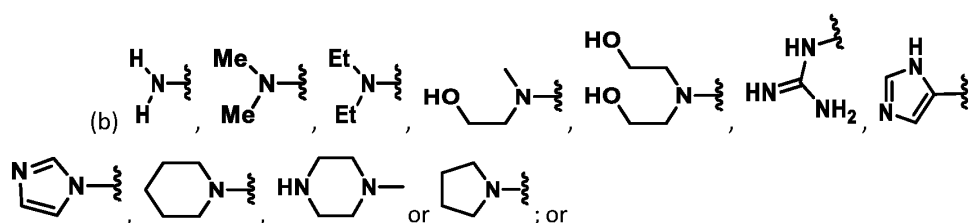
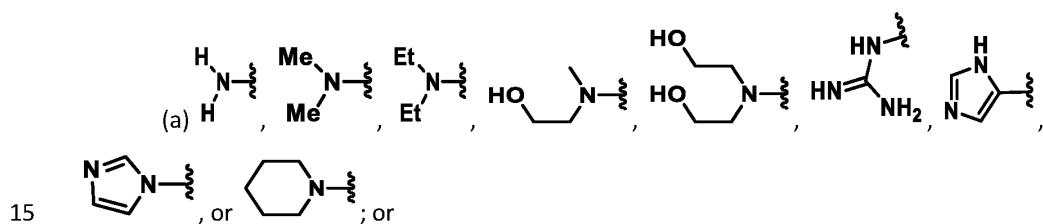
15. The compound of numbered embodiment 14, wherein B is .

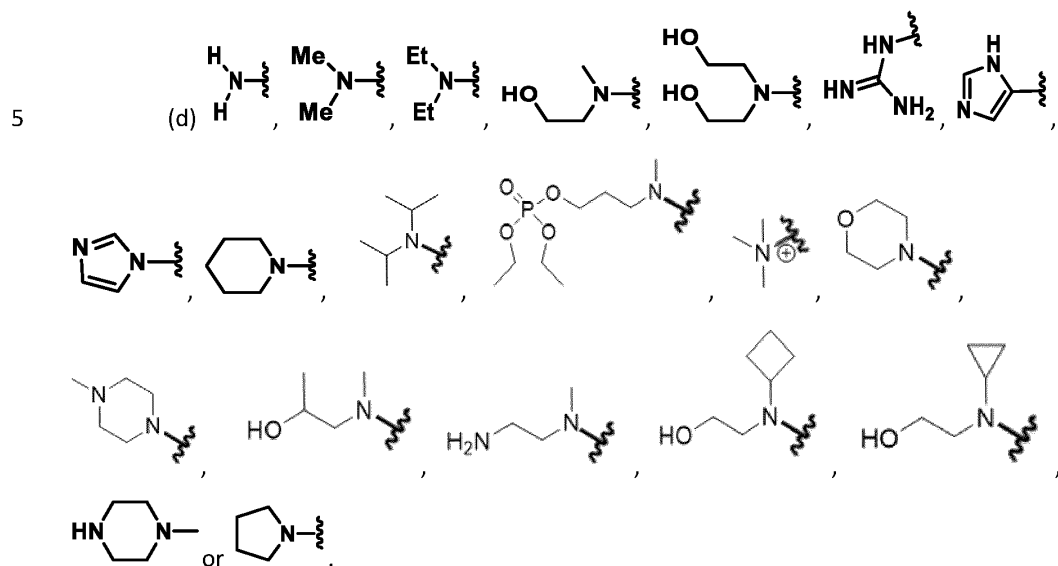
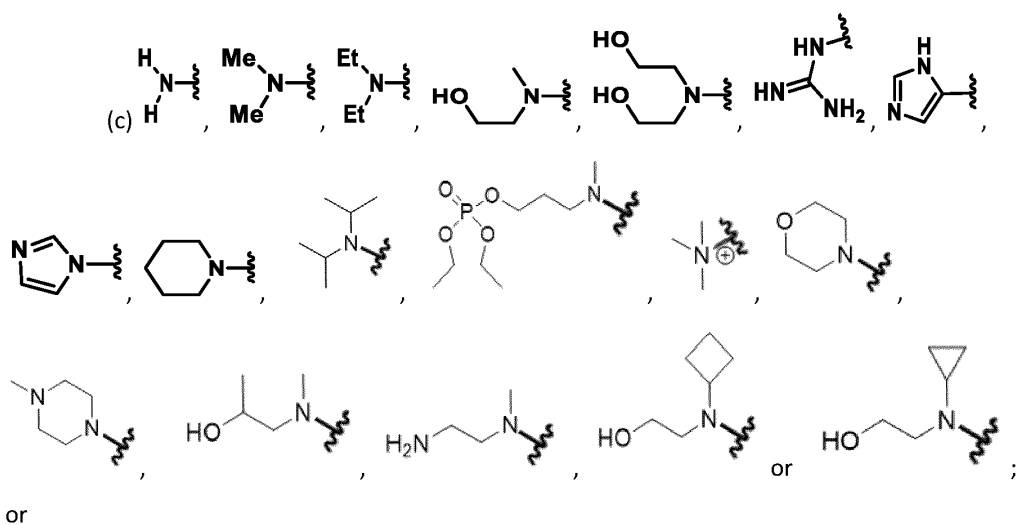
16. The compound of numbered embodiment 12, wherein said compound is Compound (3).
17. The compound of numbered embodiment 1, having a structure according to Formula (IV):



or a pharmaceutically acceptable salt thereof, wherein

- 5 each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;
- m** is an integer of 2-10;
- n** is an integer of 2-10; and
- B** is independently an ionizable nitrogen-containing group.
- 10 18. The compound of numbered embodiment 17, wherein each of **R**¹, **R**², and **R**³ is C₄-C₃₀ heteroalkyl.
19. The compound of numbered embodiment 17 or 18, wherein **m** is 2 and/or **n** is 2 or 3.
20. The compound of any one of numbered embodiments 17-19, wherein **B** is independently



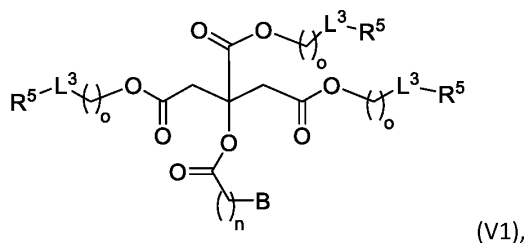


10 21. The compound of numbered embodiment 20, wherein **B** is

22. The compound of numbered embodiment 17, wherein said compound is Compound (8) or (39).

23. The compound of numbered embodiment 1, having a structure according to

(a) Formula (V1)

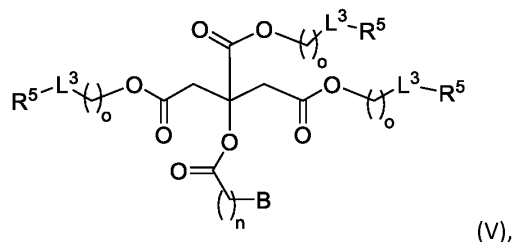


or a pharmaceutically acceptable salt thereof, wherein

5 n is an integer of 2, 3, 4, 5, 6, or 7; and

B is independently an ionizable nitrogen-containing group; or

(b) Formula (V):



or a pharmaceutically acceptable salt thereof, wherein

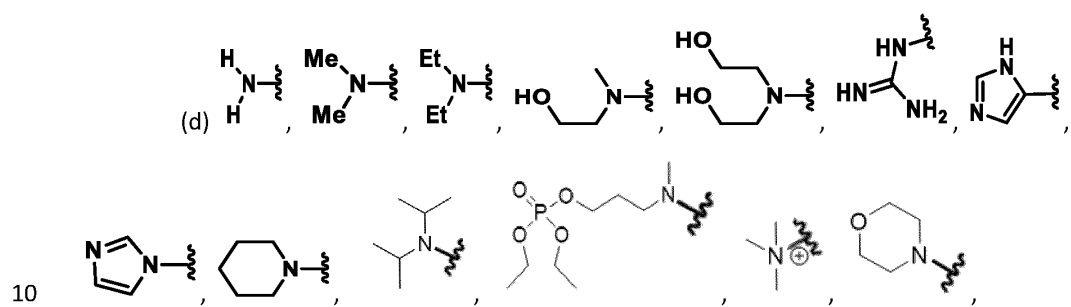
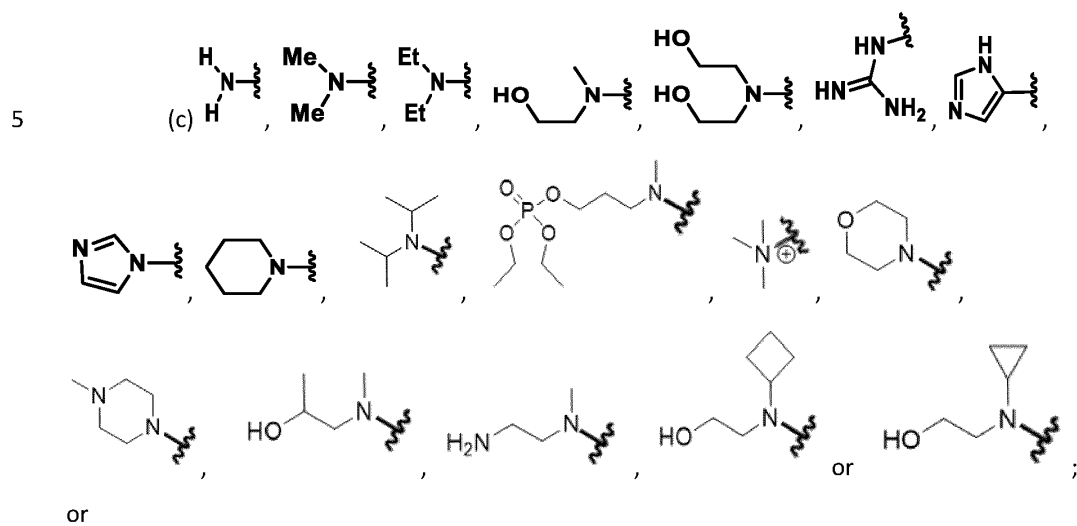
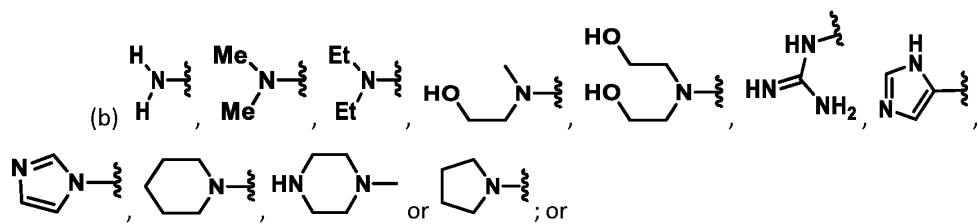
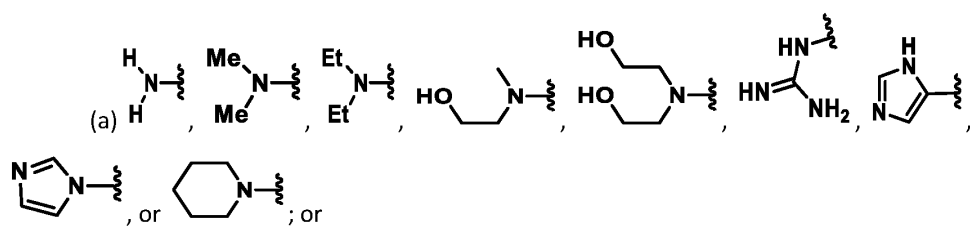
10 n is an integer of 2, 3, or 4; and

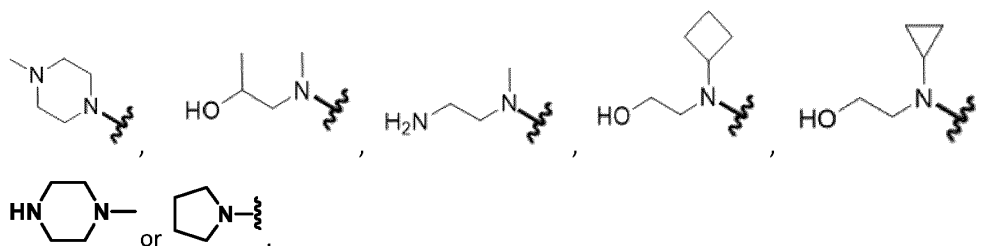
B is independently an ionizable nitrogen-containing group.

24. The compound of numbered embodiment 23, wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 2-5; and R^5 is C_4 - C_{24} alkyl.

25. The compound of numbered embodiment 23, wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 6-12; and R^5 is C_1 - C_6 alkyl; or wherein L^3 is $OC(O)$, CO_2 , or $(O)CO$; $\circ$ is an integer of 6-12; and R^5 is C_1 - C_5 alkyl.

26. The compound of any one of numbered embodiments 23-25, wherein B is independently

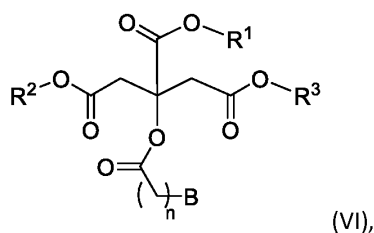




27. The compound of numbered embodiment 26, wherein **B** is

28. The compound of numbered embodiment 23, wherein said compound is selected from the group consisting of: (a) Compounds (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), and (40); or (b) Compounds (11), (12), (13), (14), (22), (25), (28), (29), (30), (32), (34), (36), (38), (40), (112), (113), (114), (115), (116), and (117).

29. The compound of numbered embodiment 1, having a structure according to Formula (VI):



10

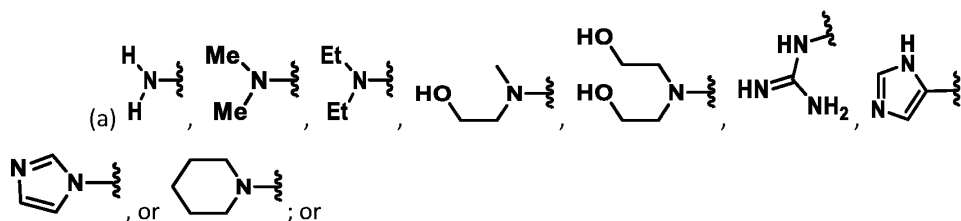
or a pharmaceutically acceptable salt thereof, wherein

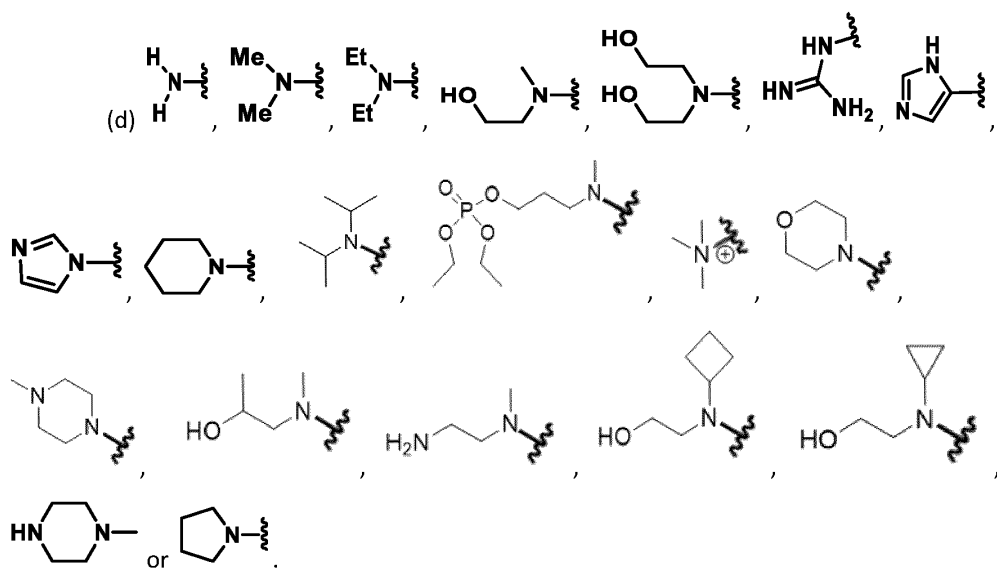
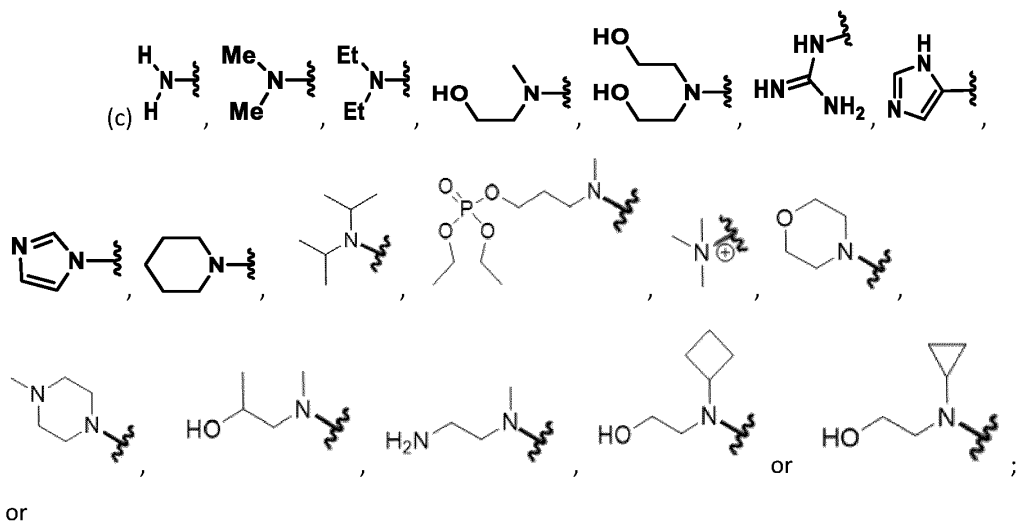
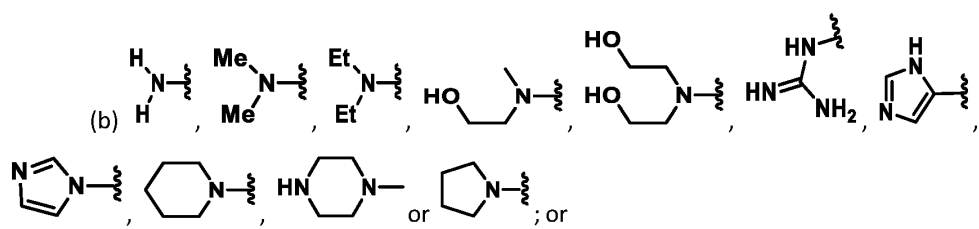
each of R^1 , R^2 , and R^3 is independently C_6-C_{30} heteroalkyl comprising a disulfide group;

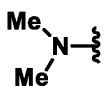
n is an integer of 2, 3, or 4; and

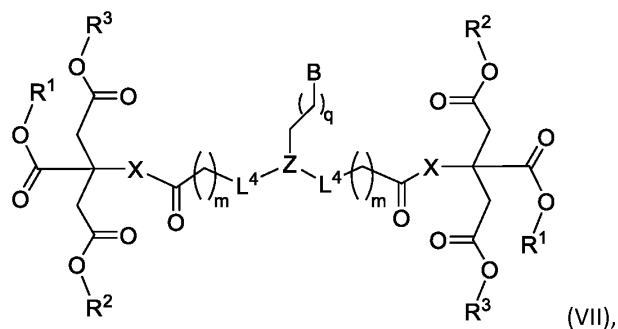
B is independently an ionizable nitrogen-containing group.

30. The compound of numbered embodiment 29, wherein **B** is independently

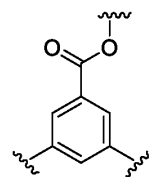




31. The compound of numbered embodiment 30, wherein **B** is .
32. The compound of numbered embodiment 29, wherein said compound is Compound (21).
33. A compound having a formula according to Formula (VII),

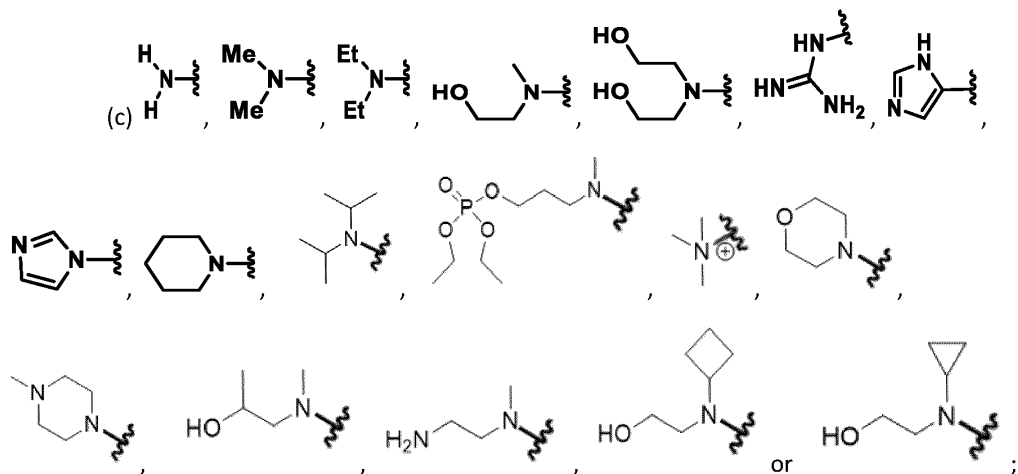
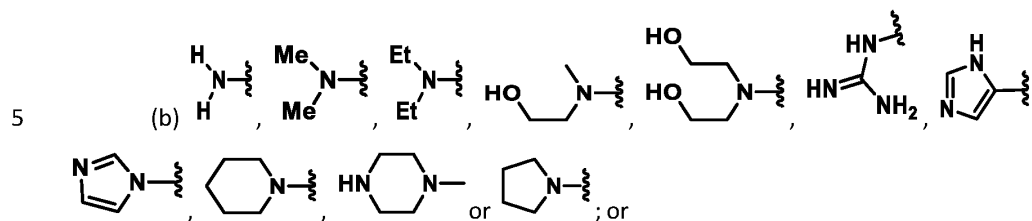
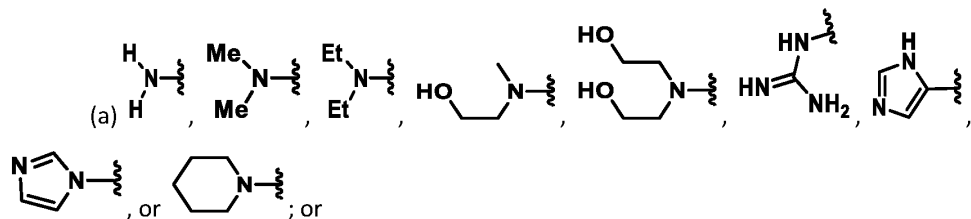


- 5 or a pharmaceutically acceptable salt thereof, wherein
- X** is independently O or NH;
- each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;
- each **m** is an integer of 2-10;
- 10 each **L⁴** is a carbonyl, ester, or amide;
- Z** is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, wherein **q1** and **q2** are independently integers of 2-10; or **Z** is C₆H₃-Z¹, wherein **Z¹** is a carbonyl, ester, or amide that is covalently attached to the $-CH_2(CH_2)_qB$ moiety;
- q** is an integer of 1-9; and
- 15 **B** is independently an ionizable nitrogen-containing group.
34. The compound of numbered embodiment 33, wherein **X** is O; each **L⁴** is $-C(O)O-$; **m** is an integer of 2, 3, or 4; and/or **q** is 1.
35. The compound of numbered embodiment 33 or 34, wherein **Z** is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, and **q1** and **q2** are each 2.

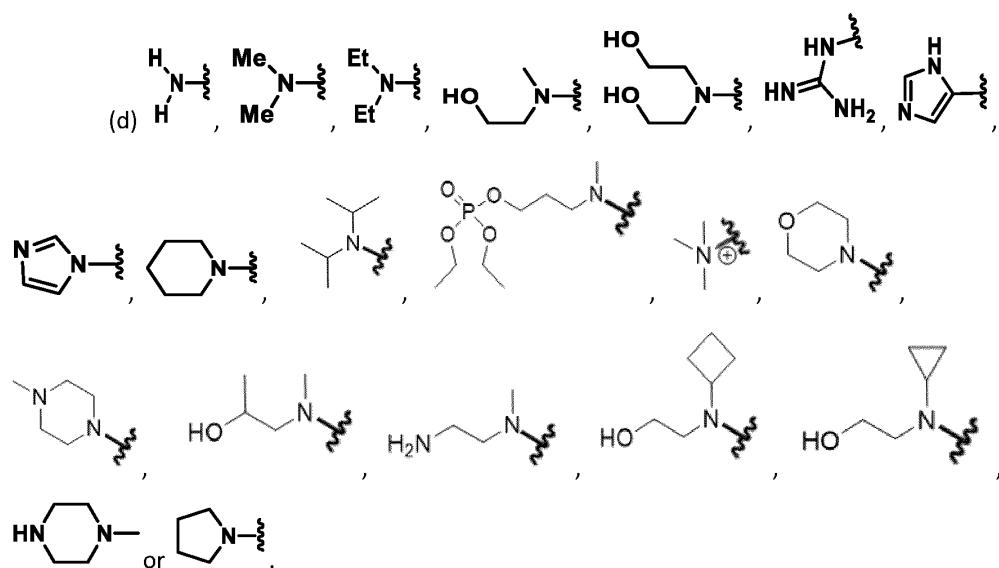


36. The compound of numbered embodiment 33 or 34, wherein **Z** is .

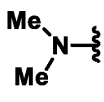
37. The compound of any one of numbered embodiments 33-36, wherein **B** is independently



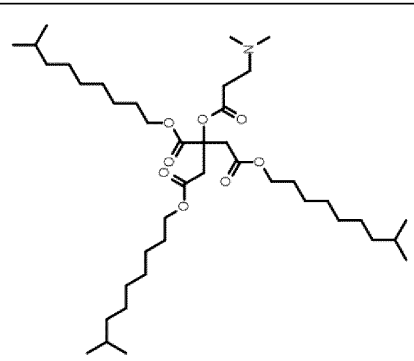
10 or

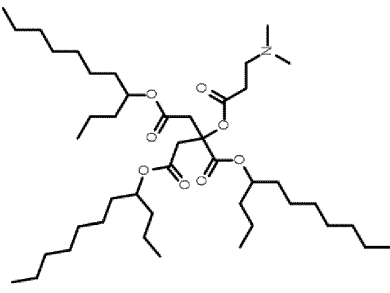
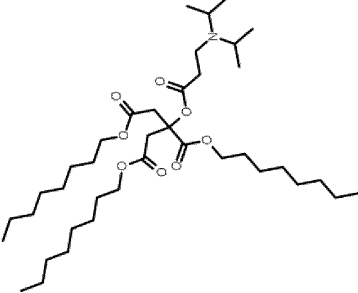
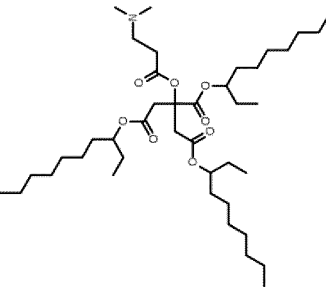
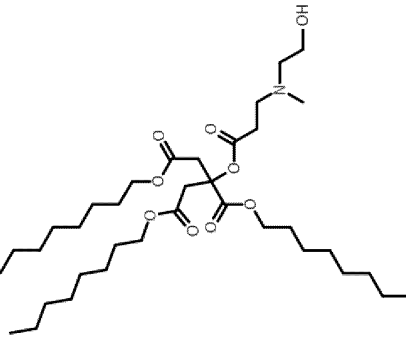


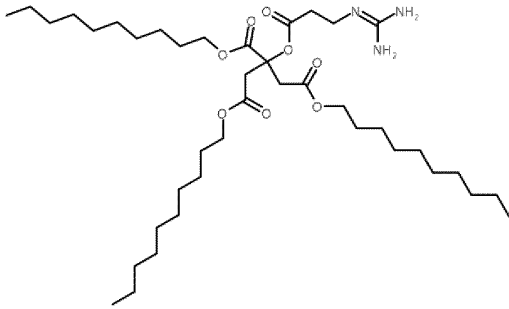
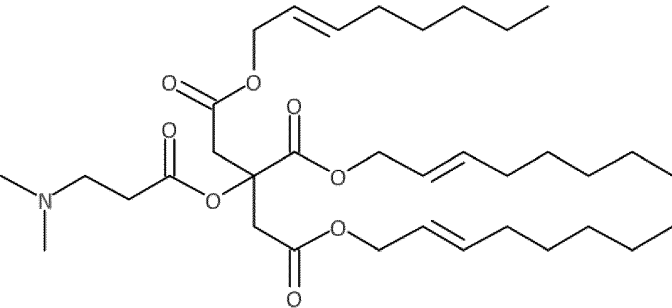
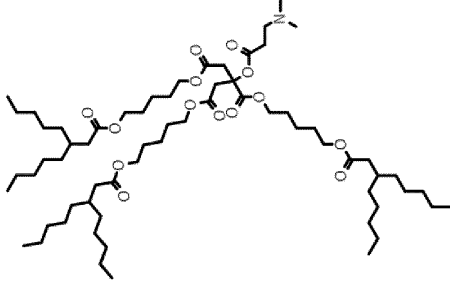
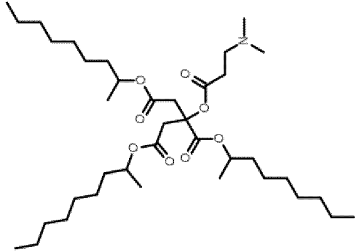
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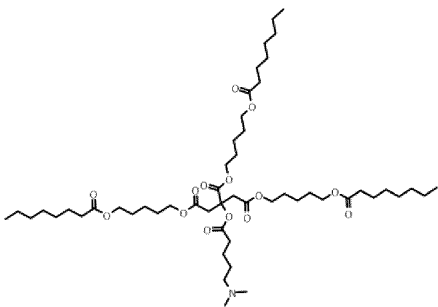
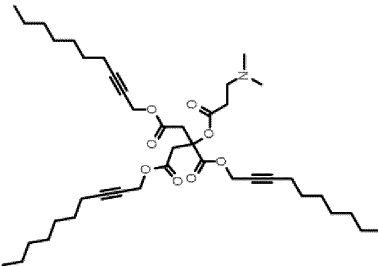
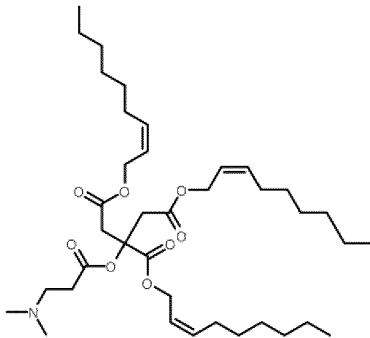
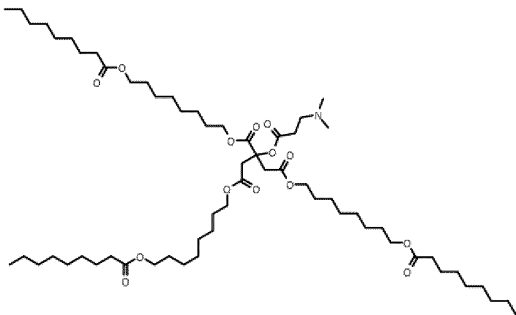
38. The compound of numbered embodiment 37, wherein **B** is .
39. The compound of numbered embodiment 33, wherein said compound is Compound (16) or (31).
40. A compound selected from the group of (a) Compounds 41-64 and 90, or a pharmaceutically acceptable salt thereof:

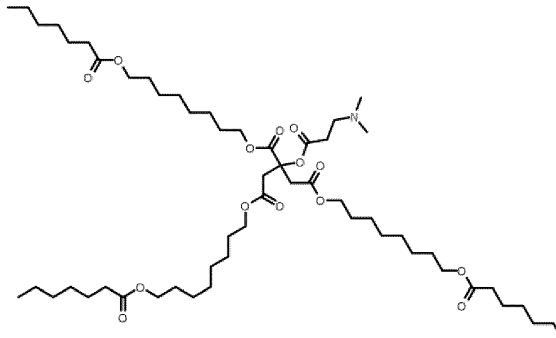
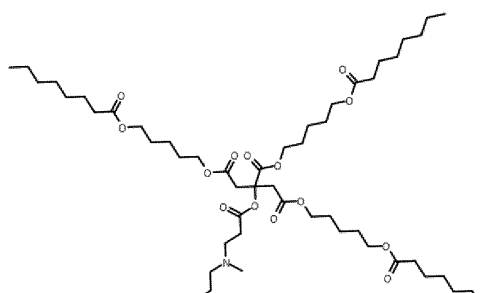
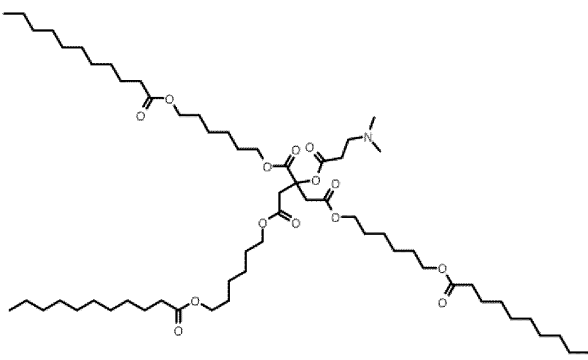
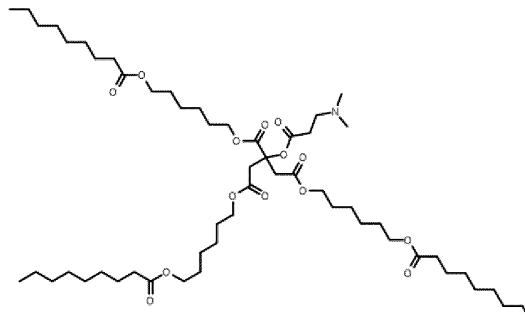
10

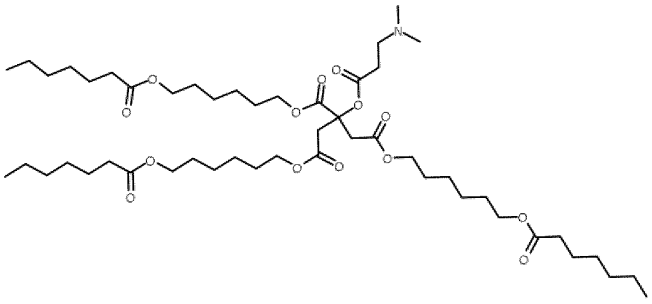
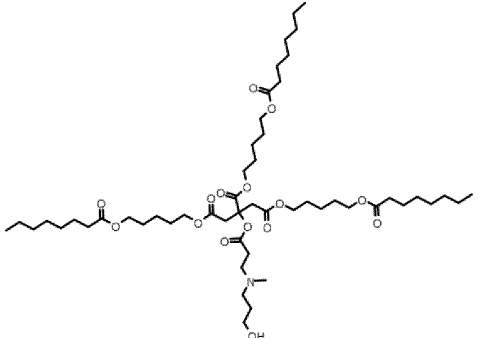
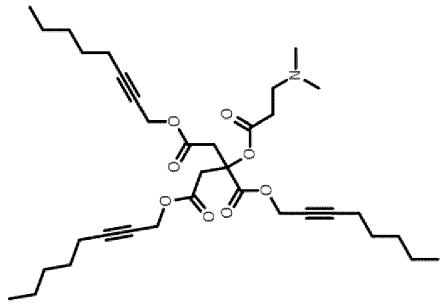
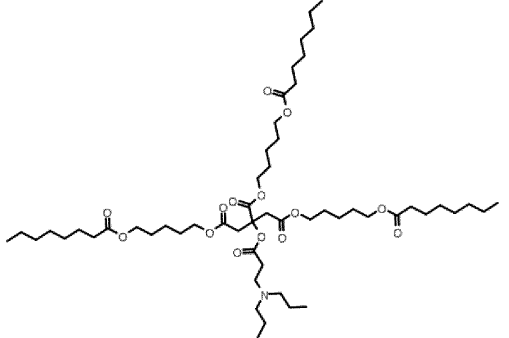
No.	Structure
41	

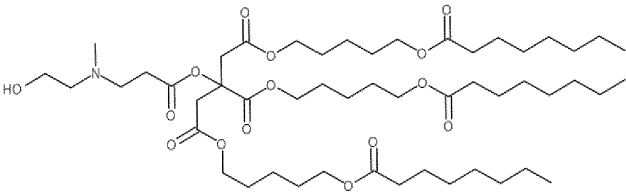
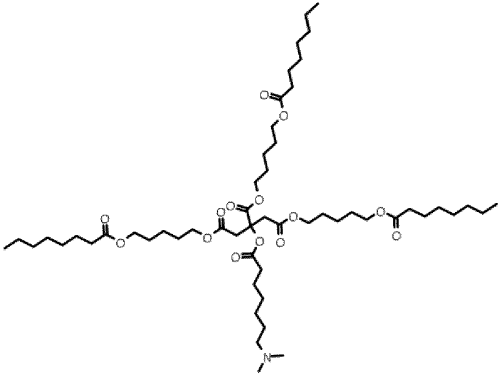
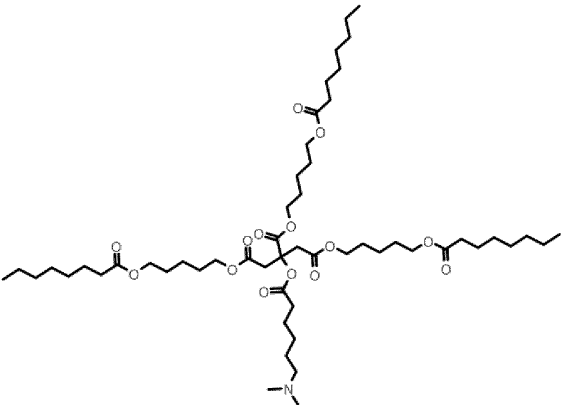
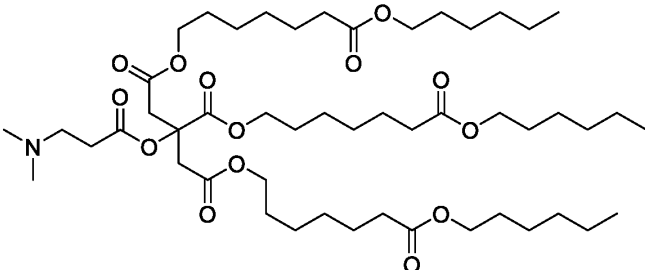
No.	Structure
42	
43	
44	
45	

No.	Structure
46	
47	
48	
49	

No.	Structure
50	
51	
52	
53	

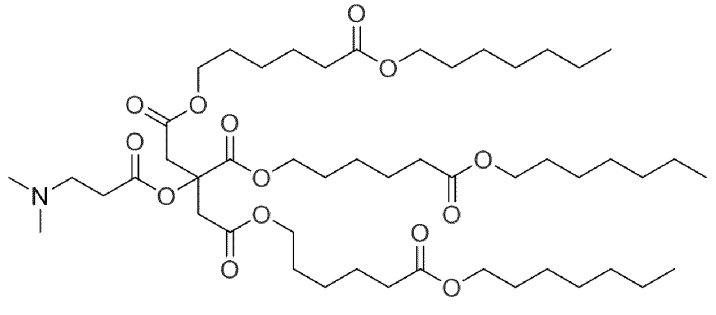
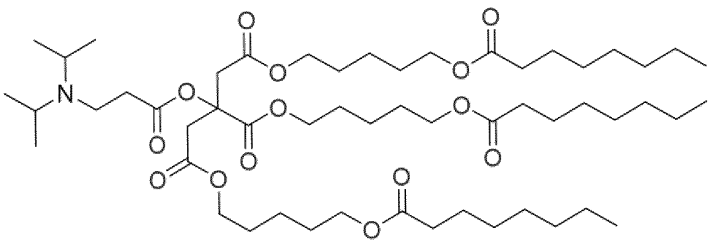
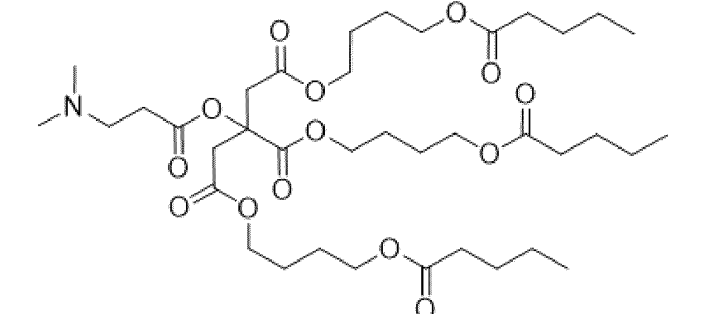
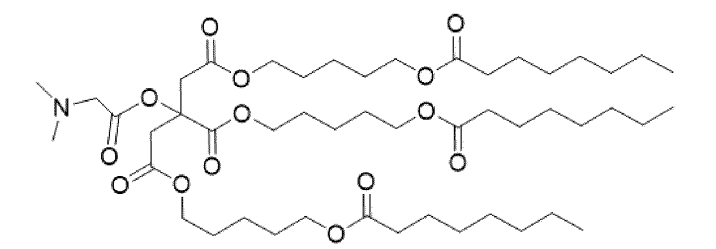
No.	Structure
54	
55	
56	
57	

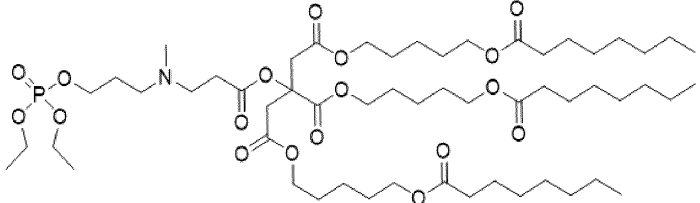
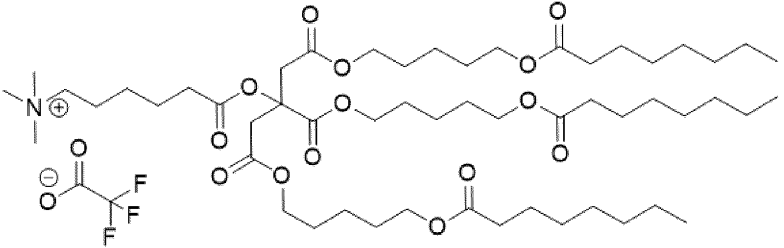
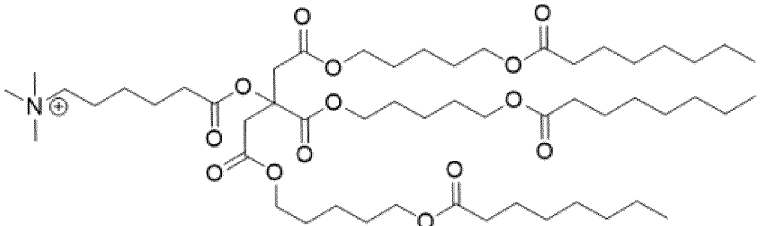
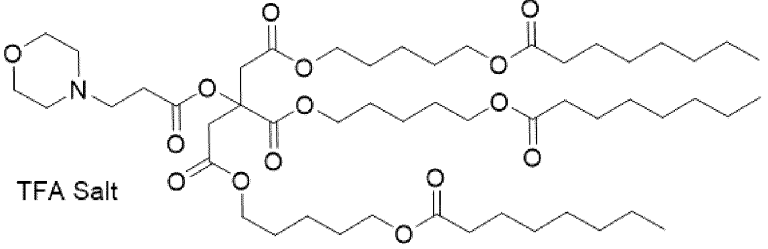
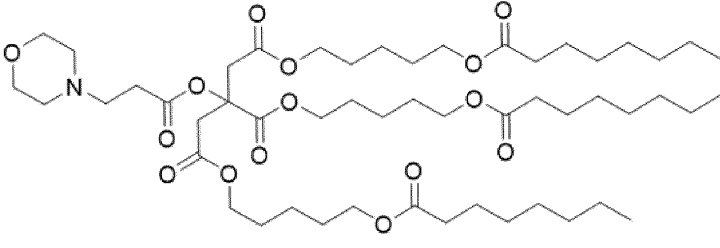
No.	Structure
58	
59	
60	
61	

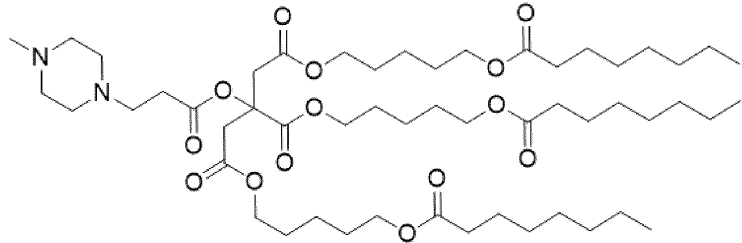
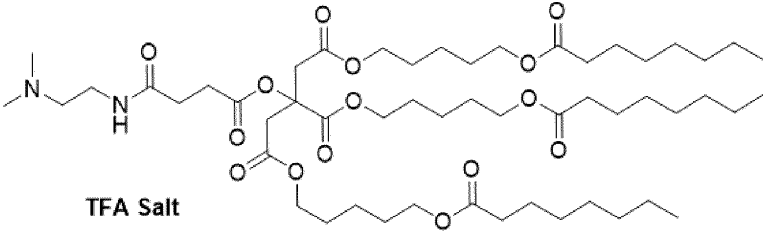
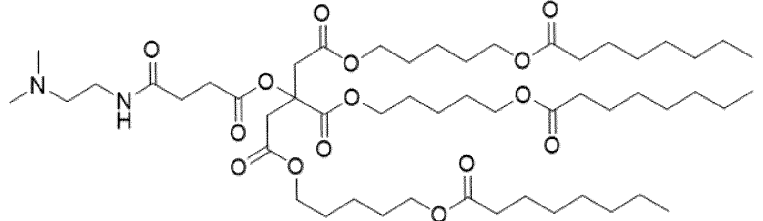
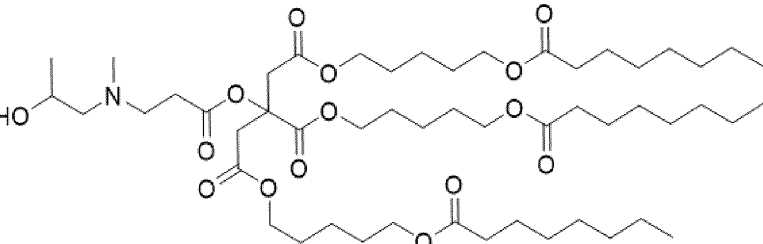
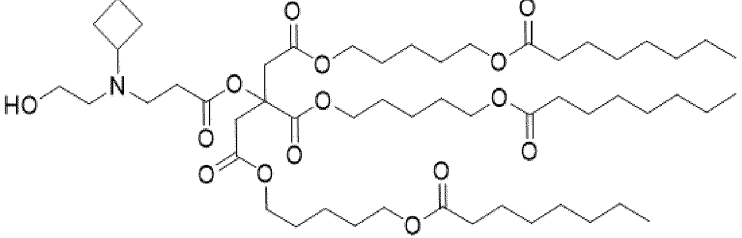
No.	Structure
62	
63	
64	
90	

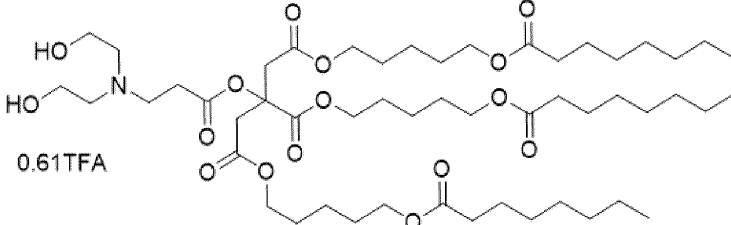
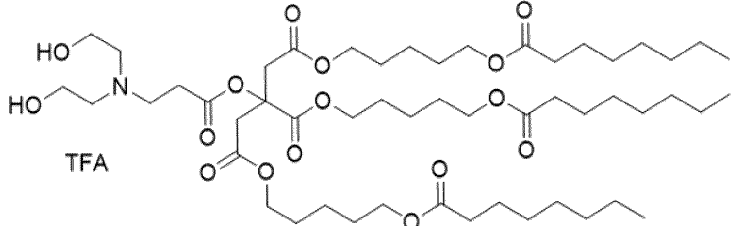
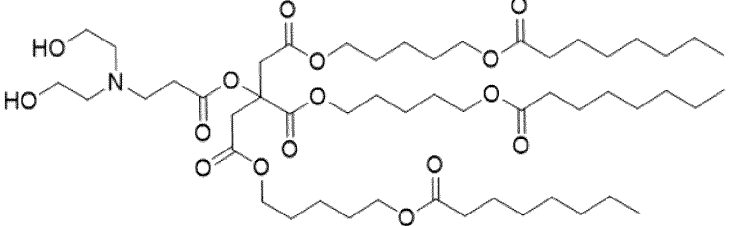
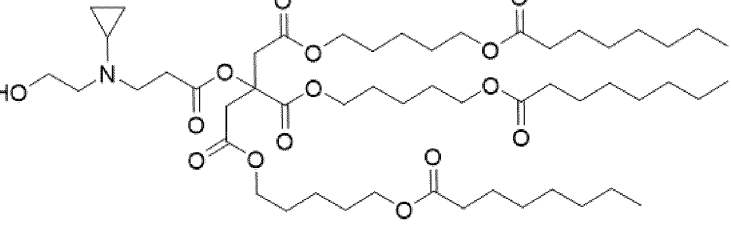
; or

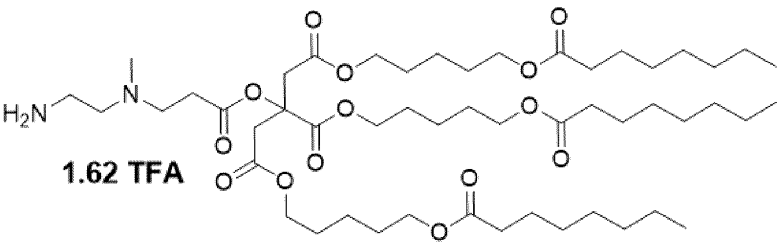
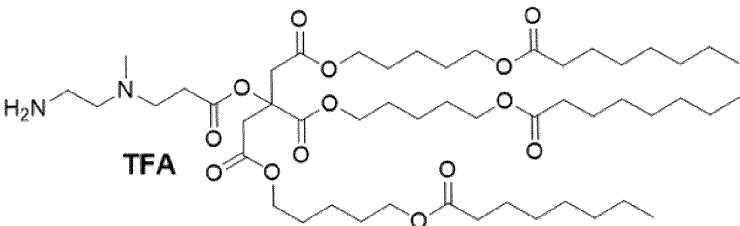
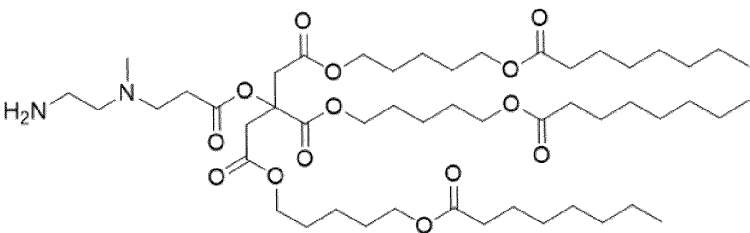
(b) Compounds 91-111, or a pharmaceutically acceptable salt thereof:

No.	Structure
91	
92	
93	
94	

No.	Structure
95	
96	
97	
98	 TFA Salt
99	

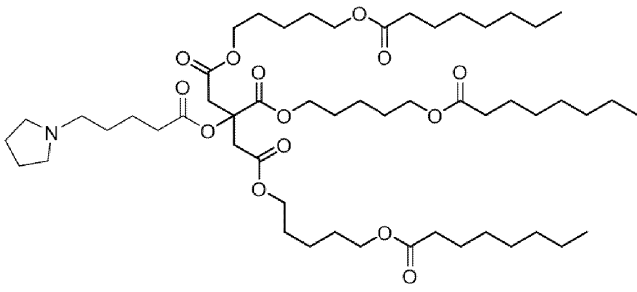
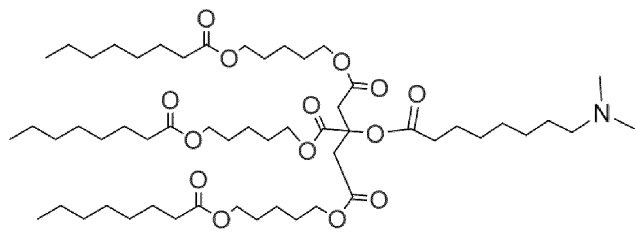
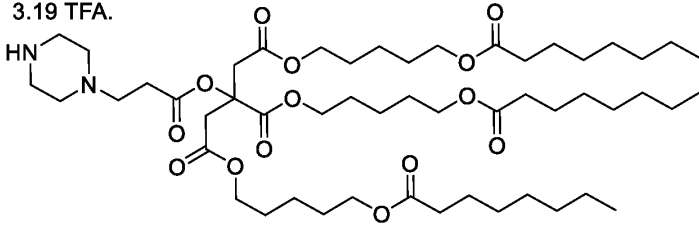
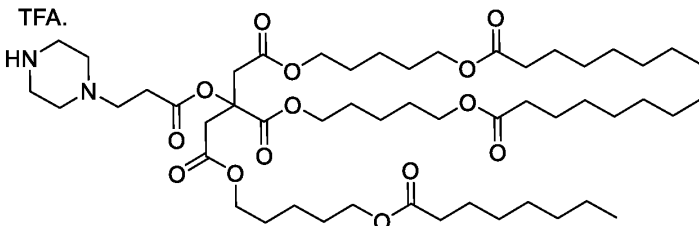
No.	Structure
100	
101	 TFA Salt
102	
103	
104	

No.	Structure
105	 0.61TFA
106	 TFA
107	
108	

No.	Structure
109	 1.62 TFA TFA content 15% (w/w)
110	 TFA
111	

(c) Compounds 112-117 or 126-129, or a pharmaceutically acceptable salt thereof:

No.	Structure
112	
113	
114	
115	

No.	Structure
116	
117	
126	3.19 TFA. 
127	TFA. 

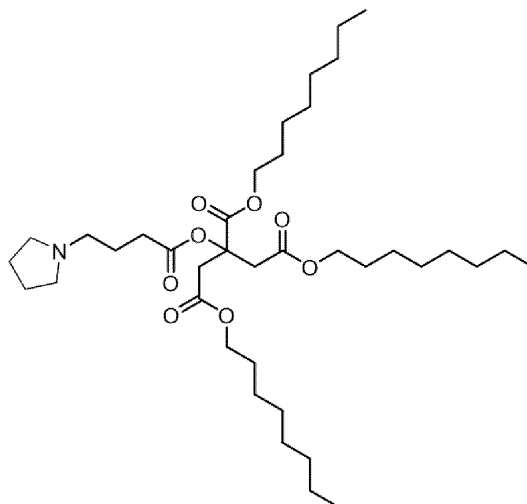
[illegible]

(d) Compounds 118-125 or a pharmaceutically acceptable salt thereof:

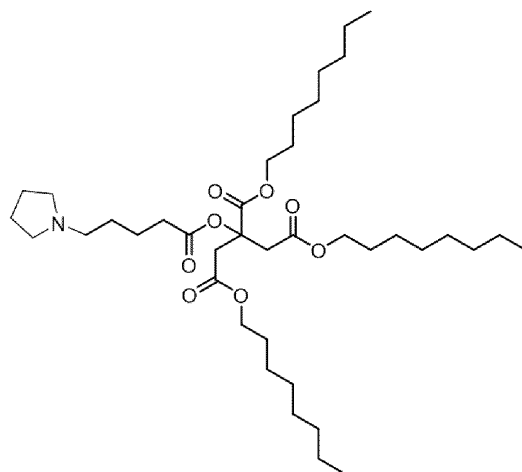
118

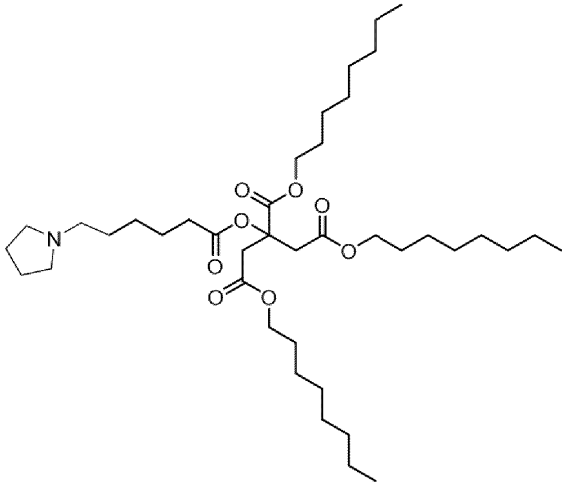
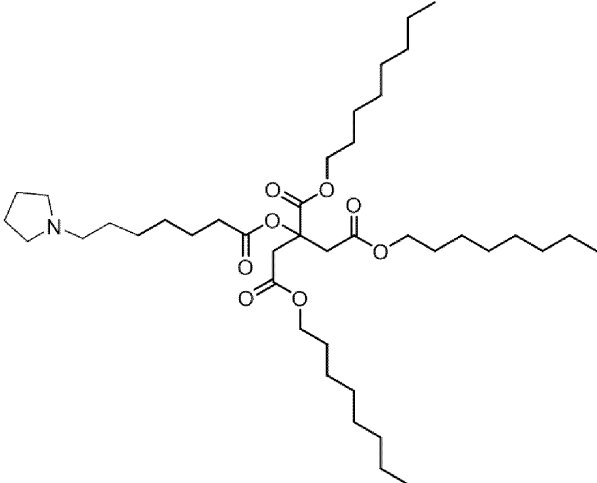
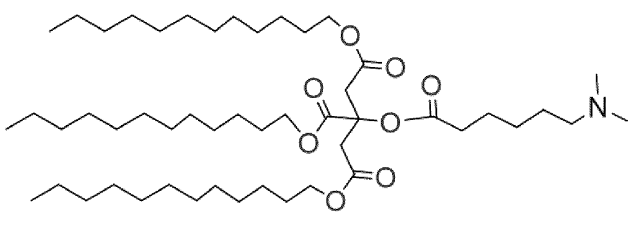
Chemical structure of a complex ester derivative, likely a surfactant or emulsifier. The structure features a central carbon atom bonded to a dimethylamino group (N(CH₃)₂), a hexyl chain, and two hexyl ester groups. The central carbon is also part of a larger ester system with a hexyl chain and a hexyl ester group.

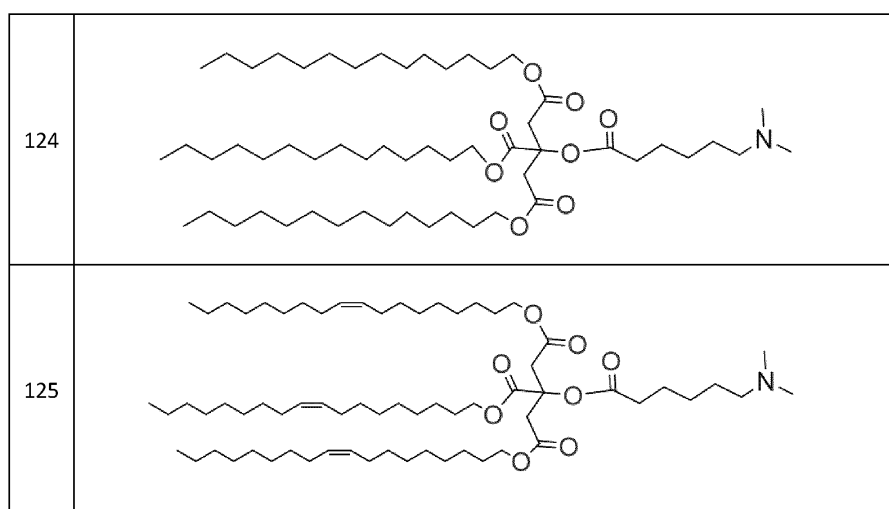
119



120



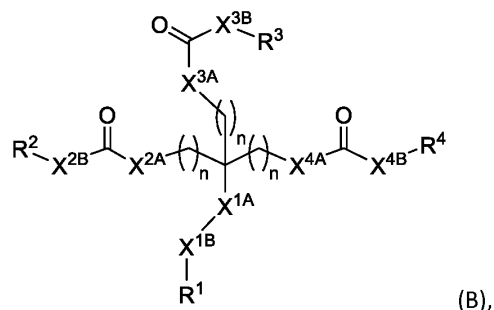
121	 <chem>CCCCCCCC(=O)OC(C)(C(=O)OCCCCC)C(=O)OCCCCC</chem>
122	 <chem>CCCCCCCC(=O)OC(C)(C(=O)OCCCCC)C(=O)OCCCCC</chem>
123	 <chem>CCCCCCCC(=O)OC(C)(C(=O)OCCCCC)C(=O)OCCCCC</chem>



41. A composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is the compound of any one of numbered embodiments 1–40.
42. A composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound of any one of numbered embodiments 1–40.
43. The composition of numbered embodiment 42, further comprising one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids.
44. The composition of numbered embodiment 42 or 43, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide.
45. The composition of any one of numbered embodiments 41–44, comprising an mRNA that encodes a peptide or polypeptide for use in vaccine.
46. The composition of numbered embodiment 45, wherein the mRNA encodes an antigen.

47. The composition of numbered embodiment 46, wherein the antigen is from an infectious agent.
48. The composition of any one of numbered embodiments 41-47, formulated for a route of administration that is oral, rectal, vaginal, transmucosal, pulmonary, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, or intranasal.
49. The composition of numbered embodiment 48, wherein said pulmonary delivery is intratracheal or inhaled.
- 10 50. The composition of numbered embodiment 48, wherein said route of administration is intranasal, optionally wherein the liposome comprises the cationic lipid compound 64, or a cationic lipid in Table 6, or a pharmaceutically acceptable salt thereof.
51. The composition of numbered embodiment 48, wherein said route of administration is intramuscular.
- 15 52. The composition of numbered embodiment 48, wherein said route of administration is pulmonary.
53. A method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids,

optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

5 each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A} ;

R^{1A} is H or C_1 - C_6 alkyl;

X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

10 one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R^1 is independently L^1 - B^1 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^2 is independently L^2 - B^2 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^3 is independently L^3 - B^3 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

15 R^4 is independently L^4 - B^4 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

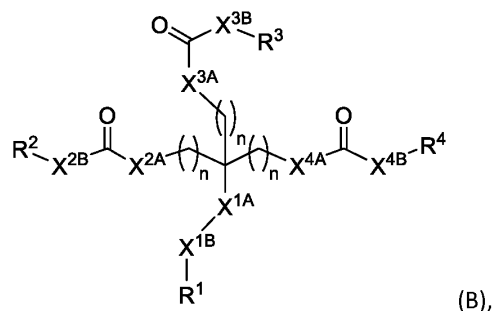
L^1 , L^2 , L^3 , and L^4 are each independently C_1 - C_{30} alkylene; C_2 - C_{30} alkenylene; or C_2 - C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

and

20 wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

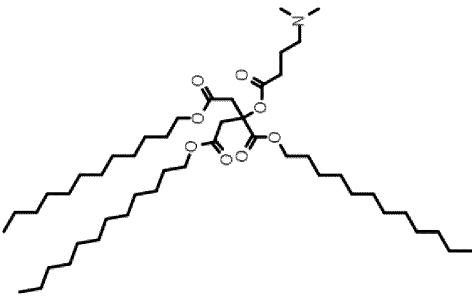
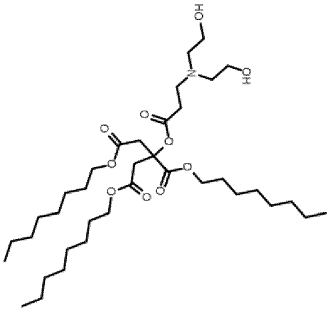
54. A method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B):

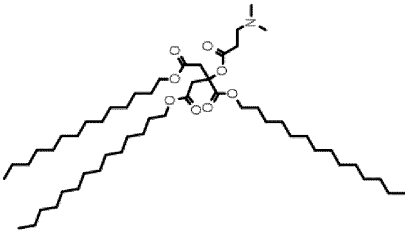
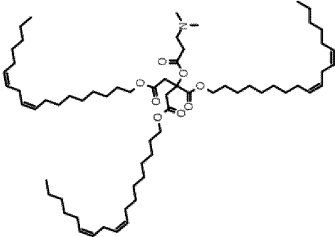
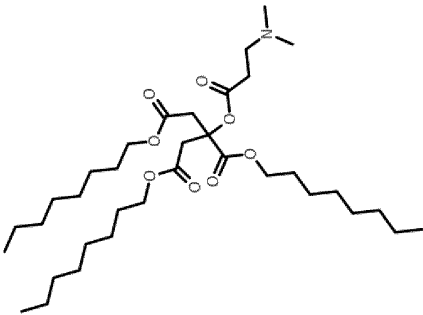
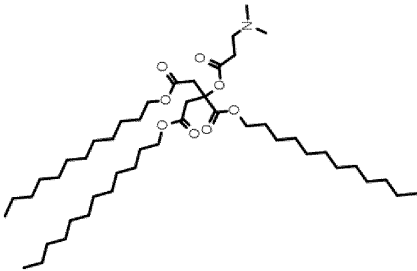


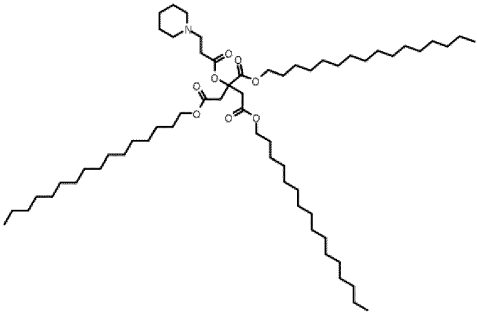
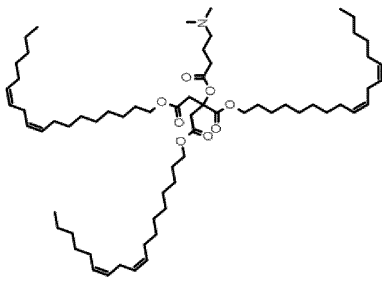
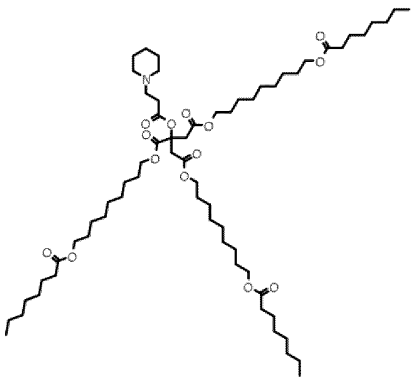
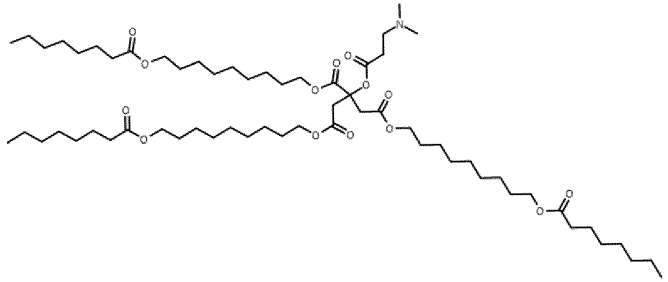
- 5 or a pharmaceutically acceptable salt thereof, wherein
 each n is independently 0 or 1;
 X^{1A} is independently O or NR^{1A} ;
 R^{1A} is H or C_1 - C_6 alkyl;
 X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;
 10 one of X^{2A} and X^{2B} is O and the other is a covalent bond;
 one of X^{3A} and X^{3B} is O and the other is a covalent bond;
 one of X^{4A} and X^{4B} is O and the other is a covalent bond;
 R^1 is independently L^1 - B^1 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 R^2 is independently L^2 - B^2 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 15 R^3 is independently L^3 - B^3 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 R^4 is independently L^4 - B^4 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;
 L^1 , L^2 , L^3 , and L^4 are each independently C_1 - C_{30} alkylene; C_2 - C_{30} alkenylene; or C_2 - C_{30} alkynylene;
 each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,
 20 and
 wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

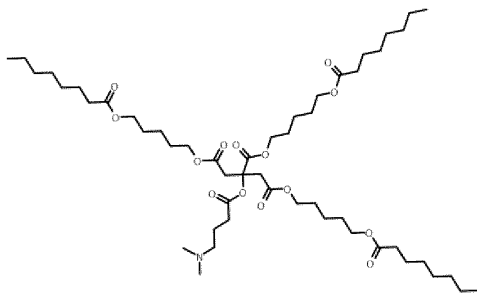
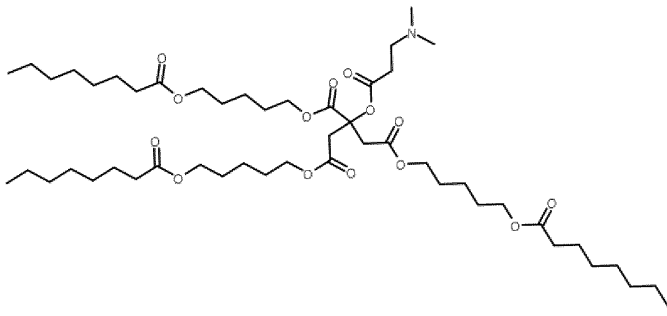
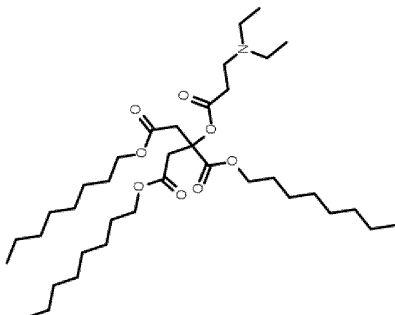
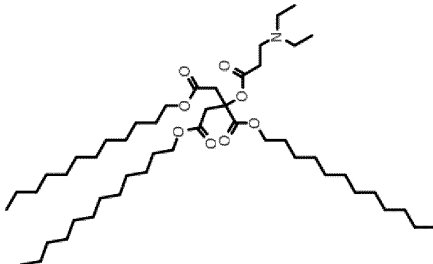
55. The method of numbered embodiment 54, wherein the composition further comprises one more lipids selected from the group consisting of one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids.

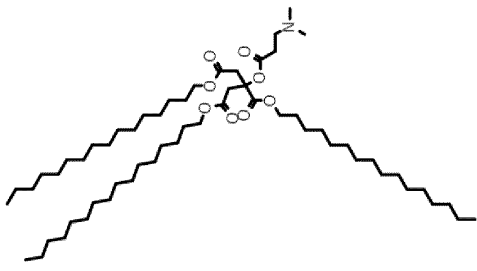
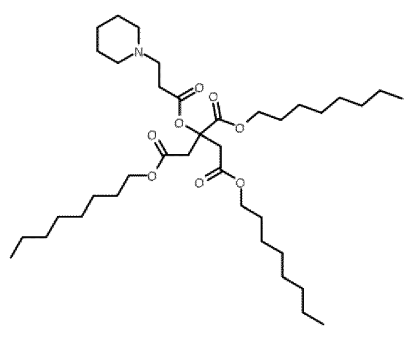
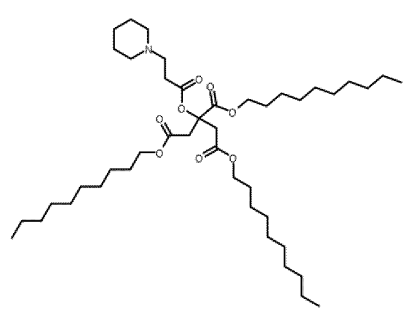
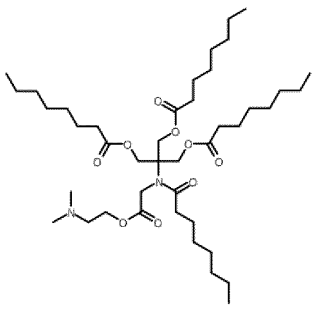
56. The method of numbered embodiment 54 or 55, wherein the nucleic acid is an mRNA encoding a peptide or polypeptide.
57. The method of any one of numbered embodiments 53–56, wherein the composition comprises an mRNA that encodes a peptide or polypeptide for use in vaccine.
- 5 58. The method of numbered embodiment 57, wherein the mRNA encodes an antigen.
59. The method of numbered embodiment 58, wherein the antigen is from an infectious agent.
60. The method of any one of numbered embodiments 53-59, wherein the compound is selected from the group consisting of (a) Compounds (65)-(89), or a pharmaceutically acceptable salt thereof:
- 10

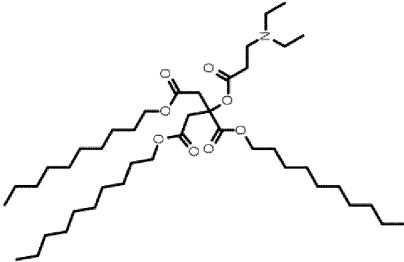
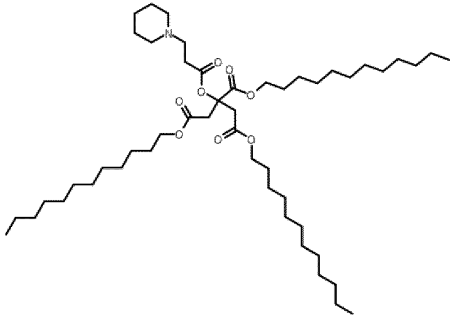
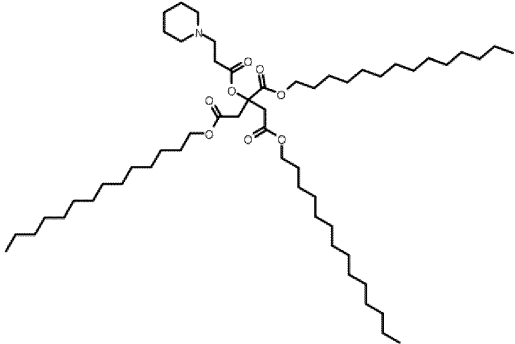
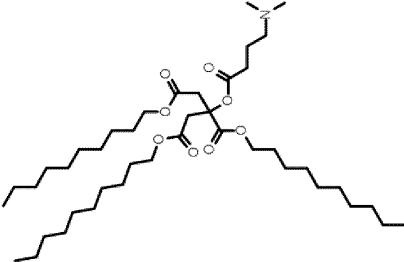
No.	Structure
65	
66	

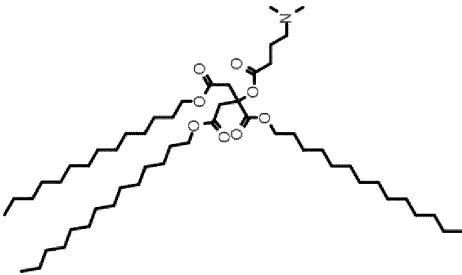
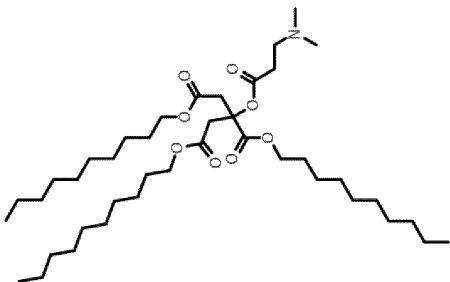
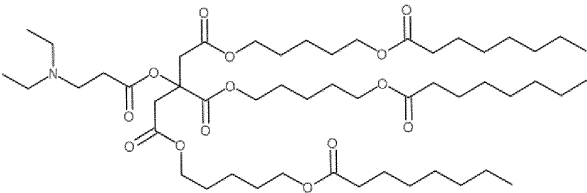
No.	Structure
67	
68	
69	
70	

No.	Structure
71	 Chemical structure 71: A central carbon atom bonded to a piperidine ring, a long alkyl chain, and two long alkyl chains via ester linkages.
72	 Chemical structure 72: A central carbon atom bonded to a long alkyl chain, a long alkyl chain, and two long alkyl chains via ester linkages.
73	 Chemical structure 73: A central carbon atom bonded to a piperidine ring, a long alkyl chain, and two long alkyl chains via ester linkages.
74	 Chemical structure 74: A central carbon atom bonded to a long alkyl chain, a long alkyl chain, and two long alkyl chains via ester linkages.

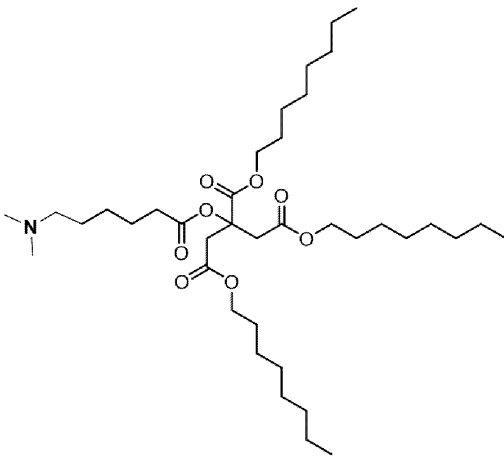
No.	Structure
75	
76	
77	
78	

No.	Structure
79	
80	
81	
82	

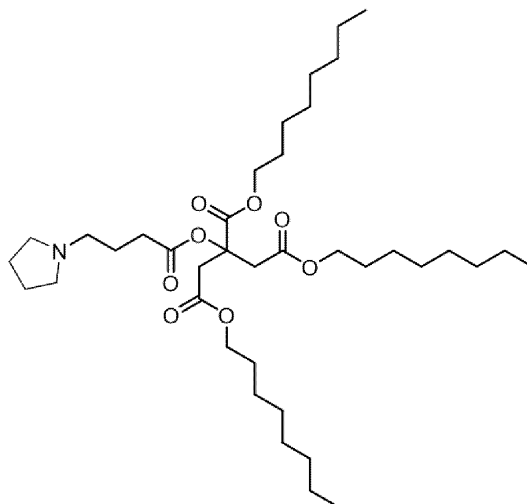
No.	Structure
83	
84	
85	
86	

No.	Structure
87	
88	
89	

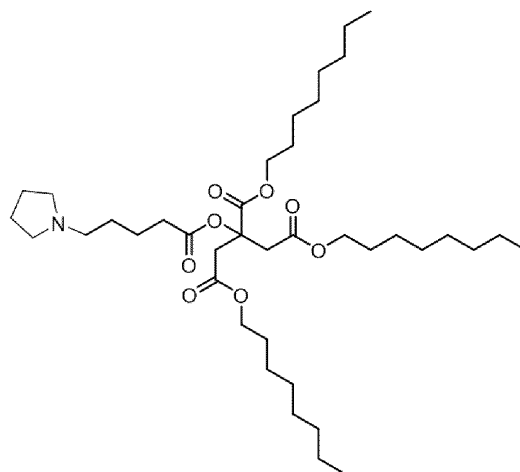
(b) Compounds 118-125, or a pharmaceutically acceptable salt thereof:

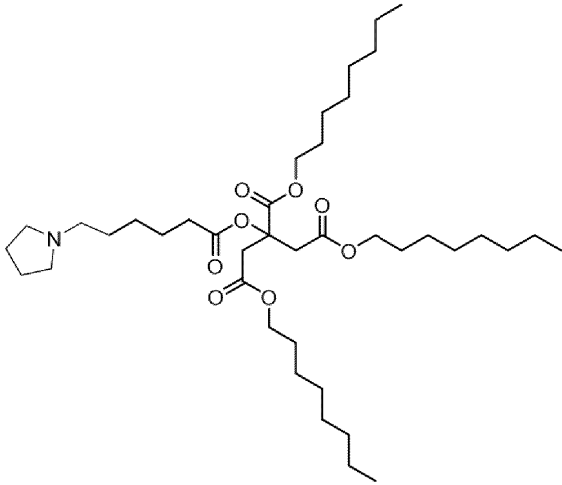
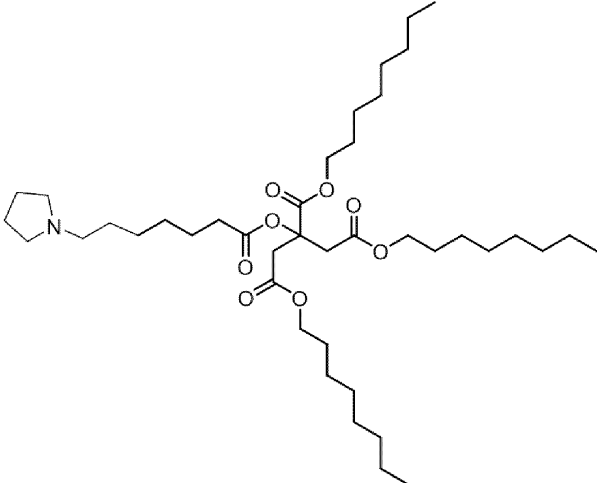
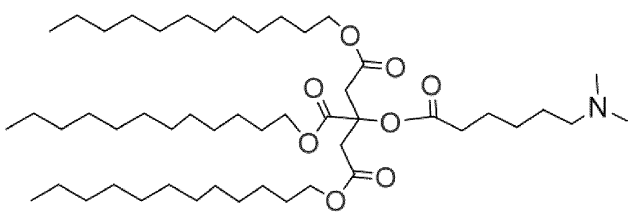
118	
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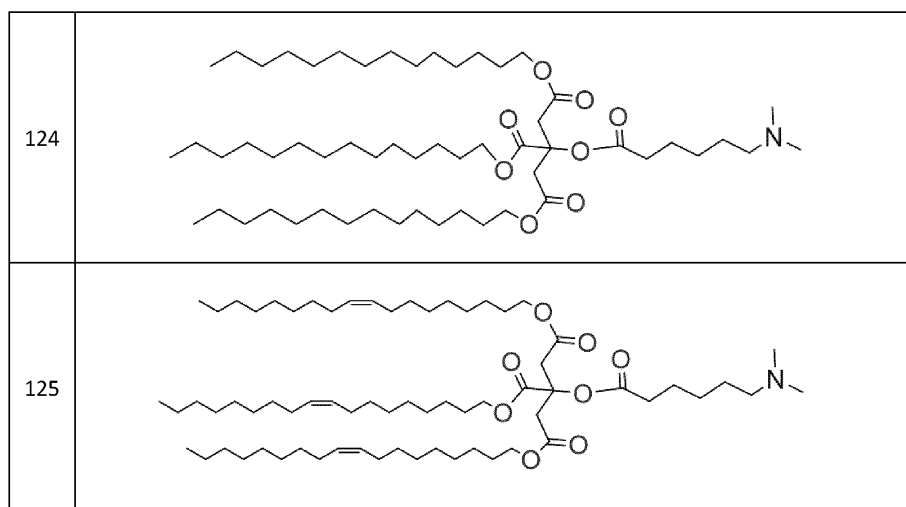
119



120



121	 <chem>CCCCCCCC(=O)OCCCCCCCC(=O)OCCCCCCCC(=O)OCCCCN1CCCC1</chem>
122	 <chem>CCCCCCCC(=O)OCCCCCCCC(=O)OCCCCCCCC(=O)OCCCCN1CCCC1</chem>
123	 <chem>CCCCCCCC(=O)OCCCCCCCC(=O)OCCCCCCCC(=O)OCCN(C)C</chem>



61. The method of any one of numbered embodiments 53-60, wherein said delivery is intranasal.
62. The method of any one of numbered embodiments 53-60, wherein said delivery is intramuscular.
63. The method of any one of numbered embodiments 53-60, wherein said delivery is pulmonary.
64. A composition comprising the cationic lipid of any one of numbered embodiments 1-40, and further comprising:
- (i) one or more non-cationic lipids,
 - (ii) one or more cholesterol-based lipids, and
 - (iii) one or more PEG-modified lipids.
65. The composition of numbered embodiment 64, wherein the composition is a lipid nanoparticle, optionally a liposome.
66. The composition of numbered embodiment 65, wherein the one or more cationic lipid(s) constitute(s) about 30 mol %-60 mol % of the lipid nanoparticle.
67. The composition of numbered embodiment 65 or 66, wherein the one or more non-cationic lipid(s) constitute(s) about 10 mol %-50 mol % of the lipid nanoparticle.
68. The composition of any one of numbered embodiments 65-67, wherein the one or more PEG-modified lipid(s) constitute(s) about 1 mol %-10 mol % of the lipid nanoparticle.

69. The composition of any one of numbered embodiments 65-68, wherein the cholesterol-based lipid constitutes about 10 mol %-50 mol% of the lipid nanoparticle.

70. The composition of any one of numbered embodiments 65-69, wherein the lipid nanoparticle encapsulates a nucleic acid, optionally an mRNA encoding a peptide or protein.

71. The composition of any one of numbered embodiments 65-70, wherein the lipid nanoparticle encapsulates an mRNA encoding a peptide or protein, optionally for use in a vaccine.

72. The composition of numbered embodiment 71, wherein the lipid nanoparticles have an encapsulation percentage for mRNA of

- (i) at least 50%;
- (ii) at least 55%;
- (iii) at least 60%;
- (iv) at least 65%;
- (v) at least 70%;
- (vi) at least 75%;
- (vii) at least 80%;
- (viii) at least 85%;
- (ix) at least 90%; or
- (x) at least 95%.

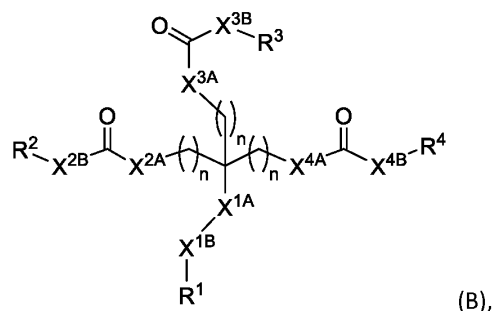
73. The composition of numbered embodiment 71 or 72 for use in therapy.

74. The composition of numbered embodiment 71 or 72 for use in a method of treating or preventing a disease amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the mRNA encodes an antigen and/or the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.

75. The composition for use according to numbered embodiment 73 or 74, wherein the composition is administered intravenously, intrathecally, intramuscularly, intranasally, sublingually, or by pulmonary delivery, optionally through nebulization.

76. The composition for use according to any one of numbered embodiments 73-75, wherein the composition is administered intranasally.
77. The composition of any one of numbered embodiments 45, 46, 47, 71, or 72, wherein the composition is for use in a method of inducing mucosal immunity, preferably wherein said mucosal immunity is induced at one or more distal mucosal sites.
78. The composition of any one of numbered embodiments 45, 46, 47, 71, or 72, wherein the composition is for use in a method of inducing a CD4 memory T cell population, optionally wherein said population is induced in the mucosa.
79. The composition for use according to numbered embodiment 77 or 78, wherein the composition is administered intravenously, intrathecally, intramuscularly, intranasally, sublingually, or by pulmonary delivery, optionally through nebulization.
80. The composition for use according to numbered embodiment 79, wherein the composition is administered intranasally.
81. A method for treating or preventing a disease wherein said method comprises administering to a subject in need thereof the composition of numbered embodiment 71 or 72 and wherein the disease is amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the mRNA encodes an antigen and/or the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.
82. A method of inducing mucosal immunity, preferably wherein said mucosal immunity is induced at one or more distal mucosal sites in a subject, wherein said method comprises administering a composition of any one of numbered embodiments 45, 46, 47, 71, or 72 to the subject.
83. A method of inducing a CD4 memory T cell population in a subject, optionally wherein said population is induced in the mucosa, wherein said method comprises administering the composition of any one of numbered embodiments 45, 46, 47, 71, or 72 to the subject.
84. The method of numbered embodiment 81, 82 or 83, wherein the composition is administered intravenously, intrathecally, intramuscularly, intranasally, sublingually, or by pulmonary delivery, optionally through nebulization.
85. The method of numbered embodiment 81 or 84, wherein the composition is administered intranasally.

86. A composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A};

R^{1A} is H or C₁-C₆ alkyl;

X^{1B} is a covalent bond, C(O), CH₂CO₂, or CH₂C(O);

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R¹ is independently L¹-B¹, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R² is independently L²-B², C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R³ is independently L³-B³, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R⁴ is independently L⁴-B⁴, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

L¹, L², L³, and L⁴ are each independently C₁-C₃₀ alkylene; C₂-C₃₀ alkenylene; or C₂-C₃₀ alkynylene;

each of B¹, B², B³, and B⁴ is independently an ionizable nitrogen-containing group,

and

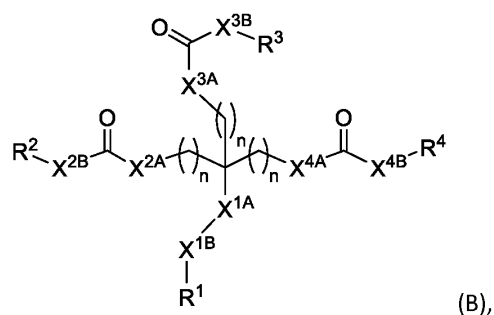
wherein the cationic lipid comprises at least one ionizable nitrogen-containing group,

wherein the composition is for use in

(a) a method of inducing mucosal immunity, preferably wherein said mucosal immunity is induced at one or more distal mucosal sites; or

(b) a method of inducing a CD4 memory T cell population, optionally wherein said population is induced in the mucosa.

87. A method of (a) of inducing mucosal immunity, preferably wherein said mucosal immunity is induced at one or more distal mucosal sites in a subject; or (b) of inducing a CD4 memory T cell population in a subject, optionally wherein said population is induced in the mucosa, wherein said method comprises administering to the subject a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A} ;

R^{1A} is H or C_1 - C_6 alkyl;

X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R^1 is independently L^1 - B^1 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^2 is independently L^2 - B^2 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^3 is independently L^3 - B^3 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^4 is independently L^4 - B^4 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

L^1 , L^2 , L^3 , and L^4 are each independently C_1 - C_{30} alkylene; C_2 - C_{30} alkenylene; or C_2 - C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

and wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

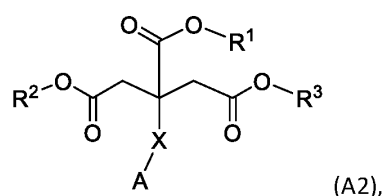
88. The composition for use according to numbered embodiment 86 or the method of numbered embodiment 87 wherein the composition is administered intravenously, intrathecally, intramuscularly, intranasally, sublingually, or by pulmonary delivery, optionally through nebulization, preferably wherein the composition is administered intranasally.

CLAIMS

WHAT IS CLAIMED IS:

1. A compound having a structure according to

Formula (A2):

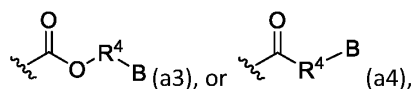
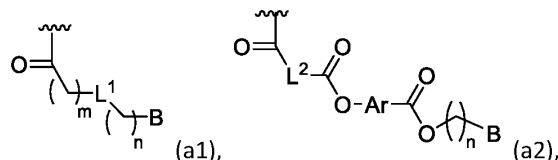


or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of R^1 , R^2 , and R^3 is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

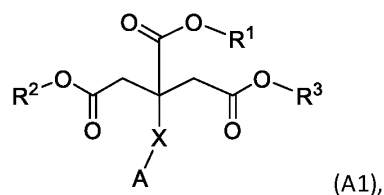
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH₃, and CH₃;

R⁴ is C₁-C₁₀ alkylene;

and wherein A is only substructure (a4) when each of R^1 , R^2 , and R^3 is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is ,
 wherein L³ is OC(O), CO₂, or (O)CO; o is an integer of 2-5; and R⁵ is C₄-C₂₄ alkyl; or L³ is OC(O), CO₂, or (O)CO; o is an integer of 6-12; and R⁵ is C₁-C₆ alkyl; or

Formula (A1):

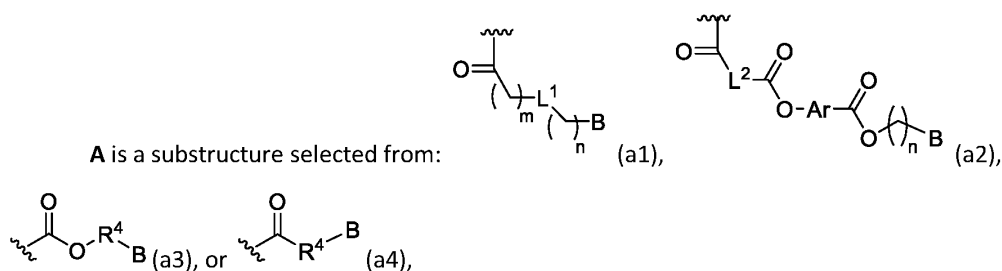


or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of **R¹**, **R²**, and **R³** is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

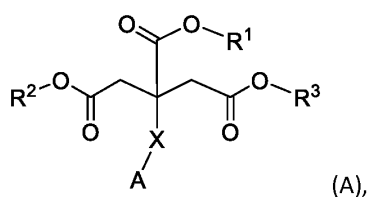
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH₃, and CH₃;

R⁴ is C₁-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R¹**, **R²**, and **R³** is independently a C₄-C₃₀

heteroalkyl comprising a disulfide bond or having a structure that is $\text{---}(\text{---})_{\text{o}}\text{---L}^3\text{---R}^5$, wherein **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R⁵** is C₄-C₂₄ alkyl; or **L³** is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R⁵** is C₁-C₅ alkyl; or

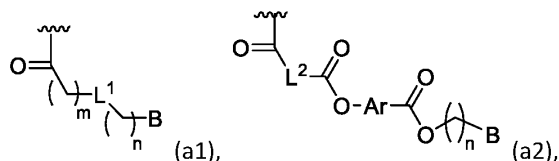
Formula (A):



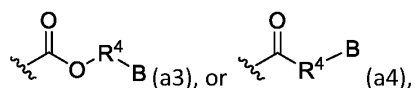
or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of **R**¹, **R**², and **R**³ is independently C₄-C₃₀ alkyl, C₄-C₃₀ alkenyl, C₄-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;



A is a substructure selected from:



B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group;

m is an integer of 2-10;

n is an integer of 2-10;

L¹ is a carbonyl, ester, or amide;

L² is C₂-C₁₀ alkylene or a C₂-C₁₀ alkenylene;

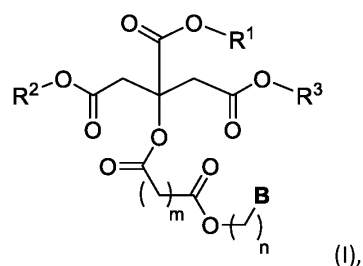
Ar is phenylene optionally comprising 1-4 substituents independently selected from halogen, OCH₃, and CH₃;

R⁴ is C₂-C₁₀ alkylene;

and wherein **A** is only substructure (a4) when each of **R**¹, **R**², and **R**³ is independently a C₄-

C₃₀ heteroalkyl comprising a disulfide bond or having a structure that is , wherein **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 2-5; and **R**⁵ is C₄-C₂₄ alkyl; or **L**³ is OC(O), CO₂, or (O)CO; **o** is an integer of 6-12; and **R**⁵ is C₁-C₅ alkyl.

2. The compound of claim 1, having a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof, wherein

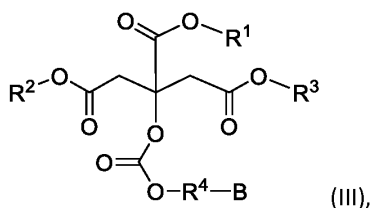
each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

m is an integer of 2-10;

n is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group or a permanently charged nitrogen group.

3. The compound of claim 1, having a structure according to Formula (III):



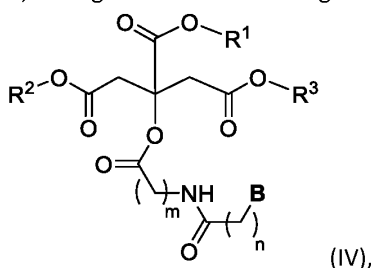
or a pharmaceutically acceptable salt thereof, wherein

each of **R**¹, **R**², and **R**³ is independently C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, C₆-C₃₀ alkynyl, or C₄-C₃₀ heteroalkyl;

R⁴ is C₂-C₁₀ alkylene; and

B is independently an ionizable nitrogen-containing group.

4. The compound of claim 1, having a structure according to Formula (IV):



or a pharmaceutically acceptable salt thereof, wherein

each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, C_6 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

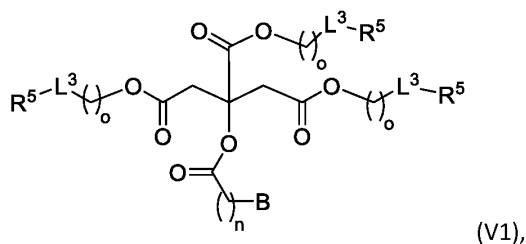
m is an integer of 2-10;

n is an integer of 2-10; and

B is independently an ionizable nitrogen-containing group.

5. The compound of claim 1, having a structure according to

(a) Formula (V1):

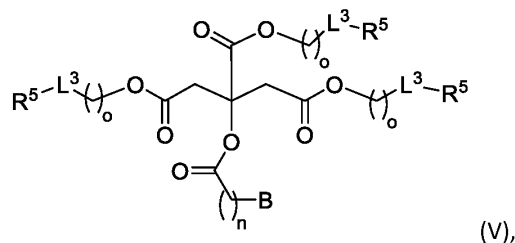


or a pharmaceutically acceptable salt thereof, wherein

n is an integer of 2, 3, 4, 5, 6, or 7; and

B is independently an ionizable nitrogen-containing group; or

(b) Formula (V):

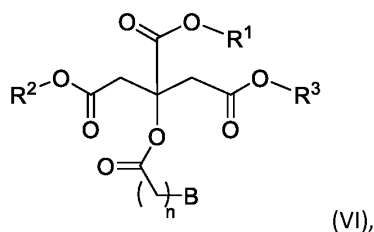


or a pharmaceutically acceptable salt thereof, wherein

n is an integer of 2, 3, or 4; and

B is independently an ionizable nitrogen-containing group.

6. The compound of claim 1, having a structure according to Formula (VI):



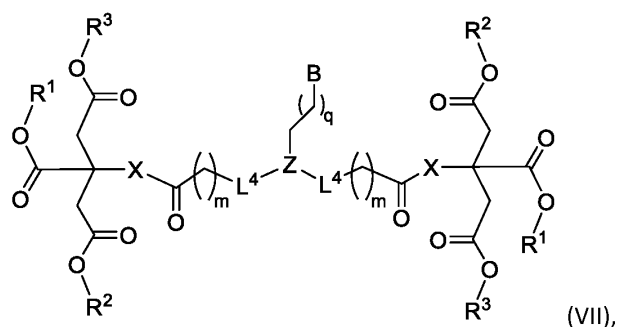
or a pharmaceutically acceptable salt thereof, wherein

each of R^1 , R^2 , and R^3 is independently C_6 - C_{30} heteroalkyl comprising a disulfide group;

n is an integer of 2, 3, or 4; and

B is independently an ionizable nitrogen-containing group.

7. A compound having a formula according to Formula (VII),



or a pharmaceutically acceptable salt thereof, wherein

X is independently O or NH;

each of R^1 , R^2 , and R^3 is independently C_4 - C_{30} alkyl, C_4 - C_{30} alkenyl, C_4 - C_{30} alkynyl, or C_4 - C_{30} heteroalkyl;

each m is an integer of 2-10;

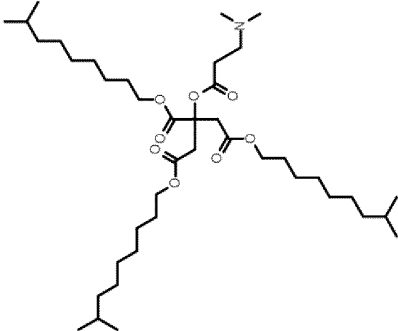
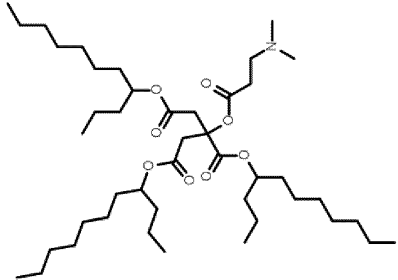
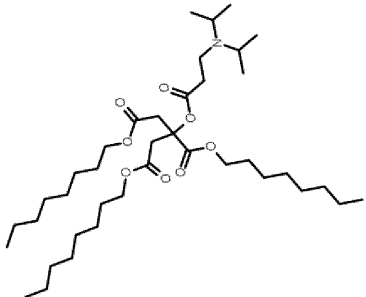
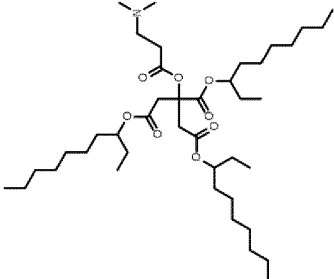
each L^4 is a carbonyl, ester, or amide;

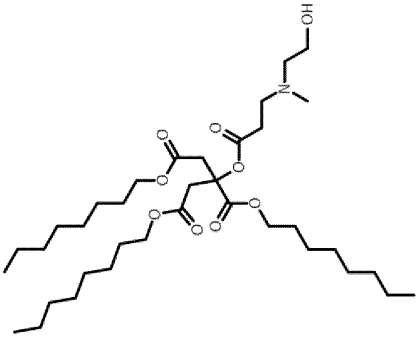
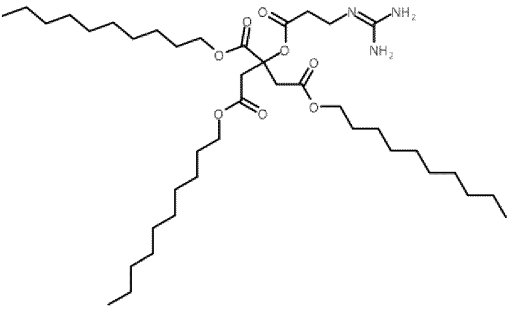
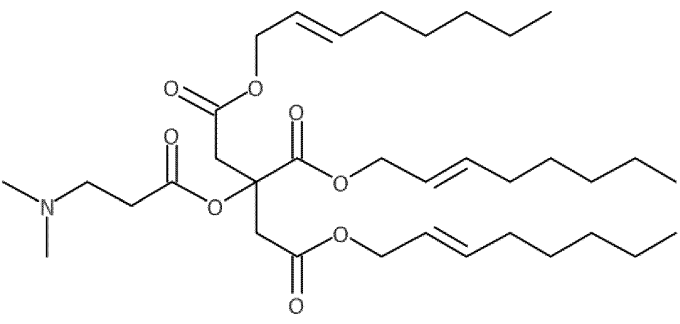
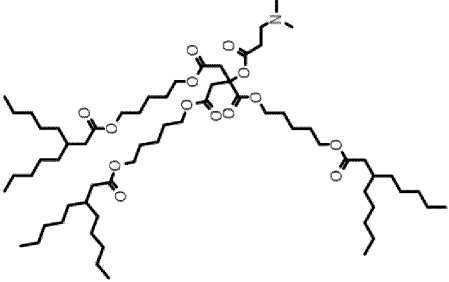
Z is $-(CH_2)_{q1}-N-(CH_2)_{q2}-$, wherein $q1$ and $q2$ are independently integers of 2-10; or Z is $C_6H_3-Z^1$, wherein Z^1 is a carbonyl, ester, or amide that is covalently attached to the $-CH_2(CH_2)_qB$ moiety;

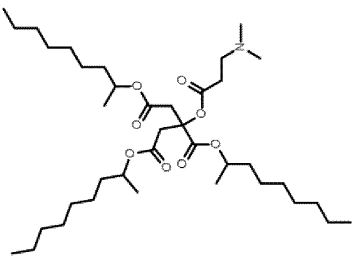
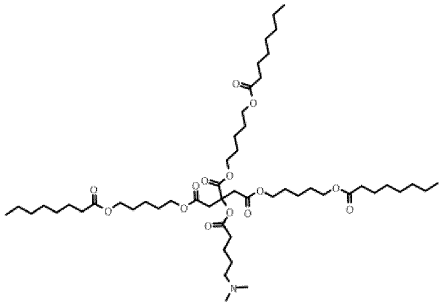
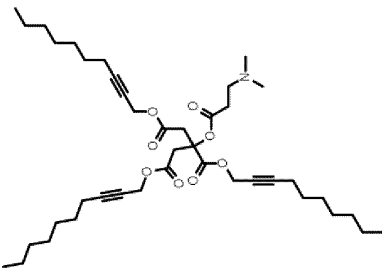
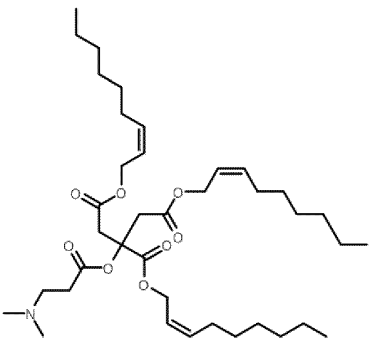
q is an integer of 1-9; and

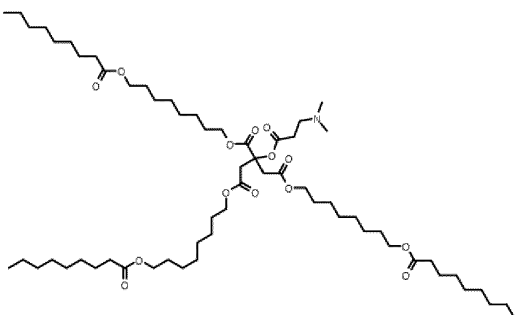
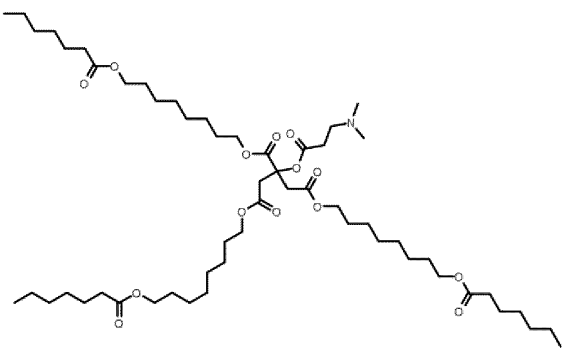
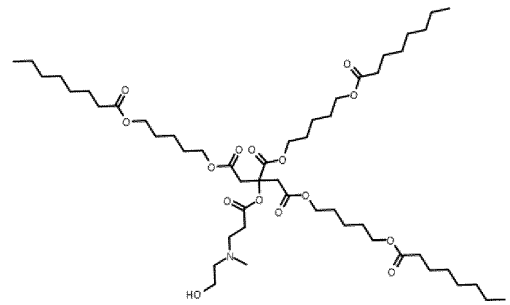
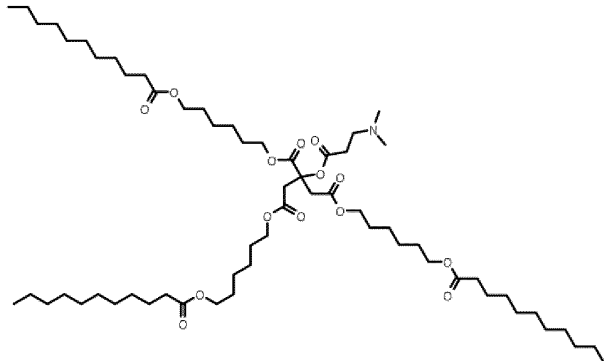
B is independently an ionizable nitrogen-containing group.

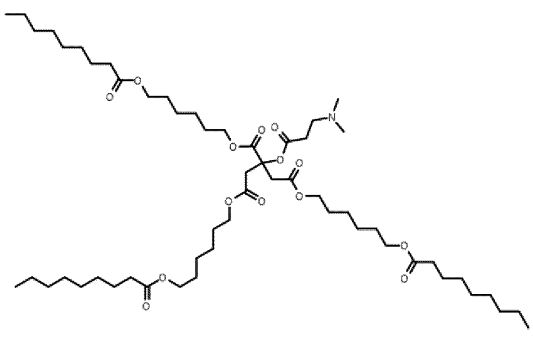
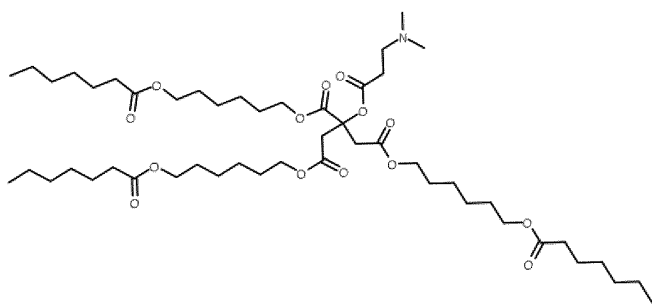
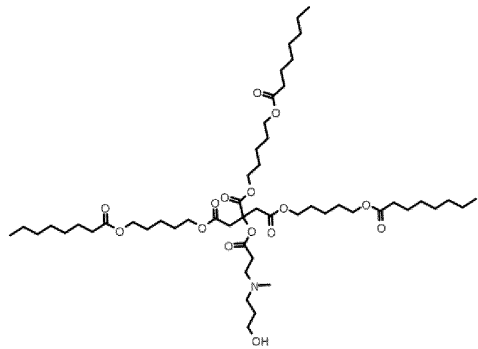
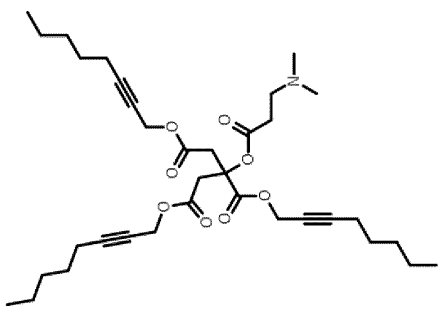
8. A compound selected from the group of (a) Compounds 41-64 and 90, or a pharmaceutically acceptable salt thereof:

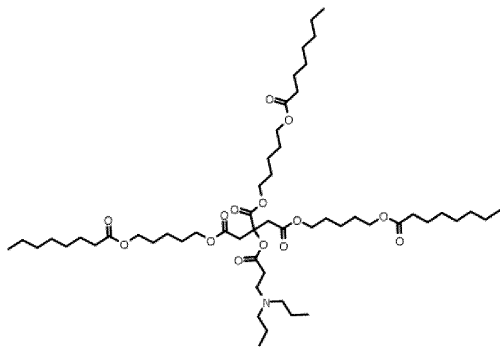
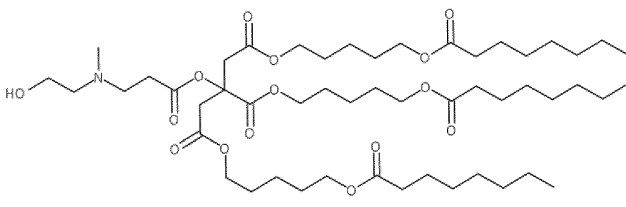
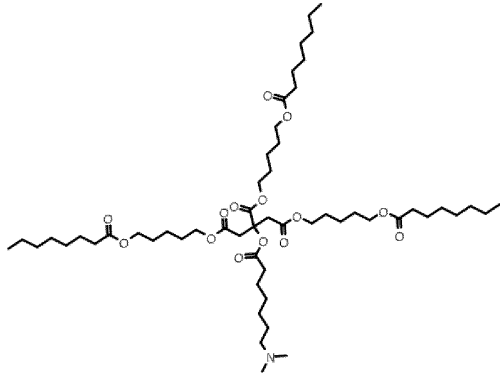
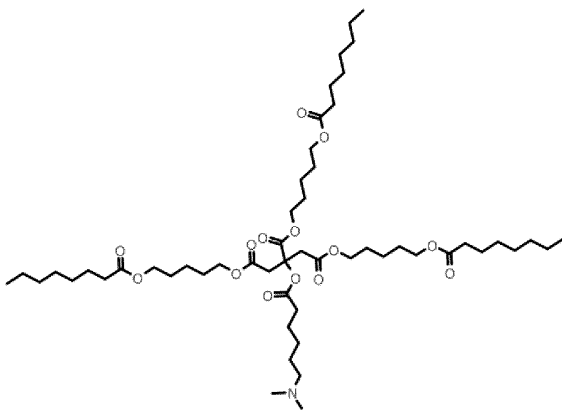
No.	Structure
41	
42	
43	
44	

No.	Structure
45	
46	
47	
48	

No.	Structure
49	
50	
51	
52	

No.	Structure
53	 Chemical structure 53: A central core with four long alkyl chains and a dimethylamino group. The structure is a complex molecule with a central core and four long alkyl chains. The chains are connected to the core via ester linkages. The central core contains a dimethylamino group (N(CH₃)₂) and several oxygen atoms. The alkyl chains are long and branched, with some containing ester groups.
54	 Chemical structure 54: A central core with four long alkyl chains and a dimethylamino group. The structure is a complex molecule with a central core and four long alkyl chains. The chains are connected to the core via ester linkages. The central core contains a dimethylamino group (N(CH₃)₂) and several oxygen atoms. The alkyl chains are long and branched, with some containing ester groups.
55	 Chemical structure 55: A central core with four long alkyl chains and a dimethylamino group. The structure is a complex molecule with a central core and four long alkyl chains. The chains are connected to the core via ester linkages. The central core contains a dimethylamino group (N(CH₃)₂) and several oxygen atoms. The alkyl chains are long and branched, with some containing ester groups. A hydroxyl group (HO) is also present on one of the chains.
56	 Chemical structure 56: A central core with four long alkyl chains and a dimethylamino group. The structure is a complex molecule with a central core and four long alkyl chains. The chains are connected to the core via ester linkages. The central core contains a dimethylamino group (N(CH₃)₂) and several oxygen atoms. The alkyl chains are long and branched, with some containing ester groups.

No.	Structure
57	
58	
59	
60	

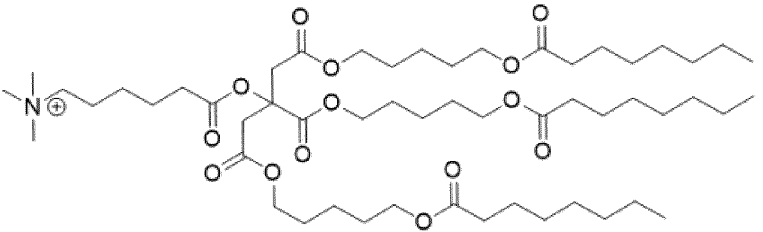
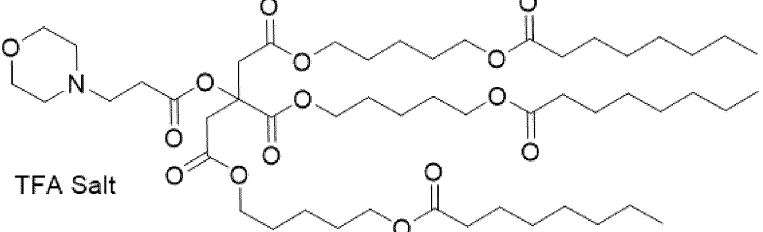
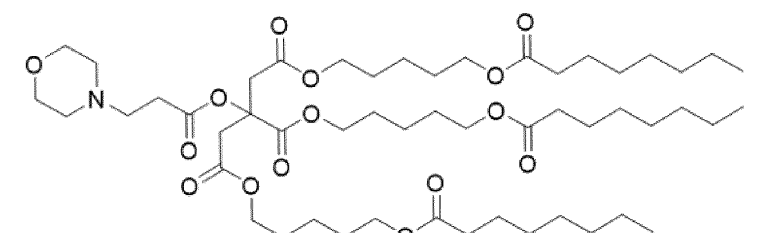
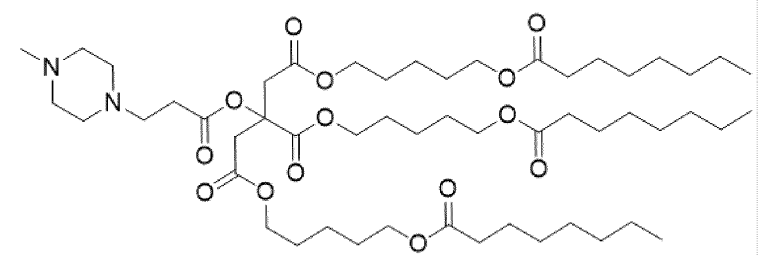
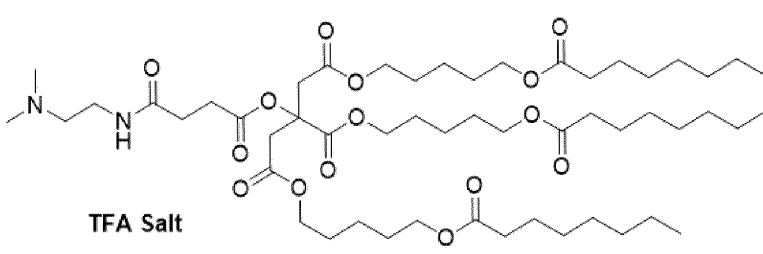
No.	Structure
61	
62	
63	
64	

No.	Structure
90	; or

(b) Compounds 91-111, or a pharmaceutically acceptable salt thereof:

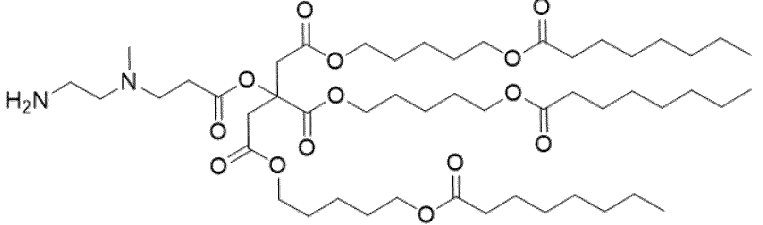
No.	Structure
91	
92	

No.	Structure
93	
94	
95	
96	

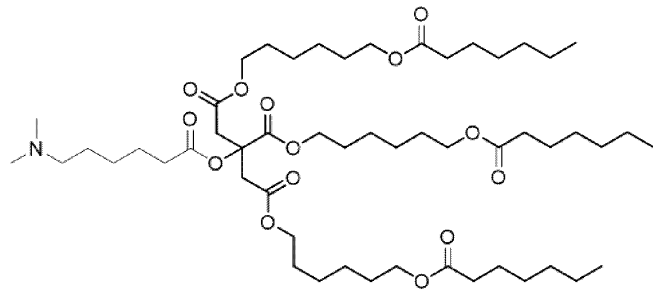
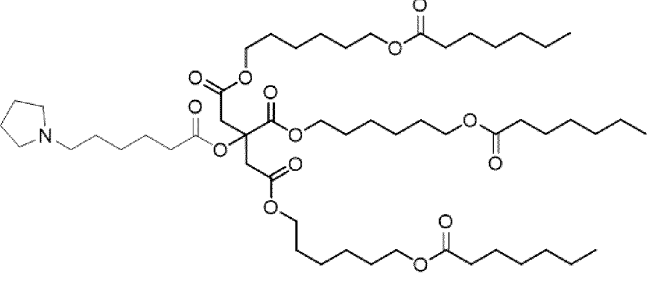
No.	Structure
97	
98	 TFA Salt
99	
100	
101	 TFA Salt

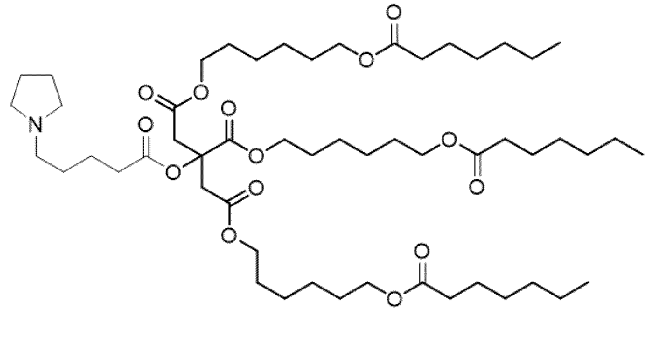
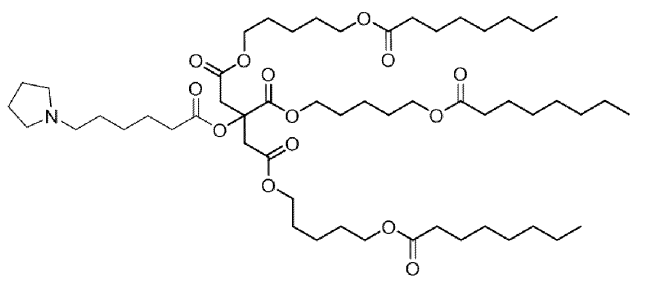
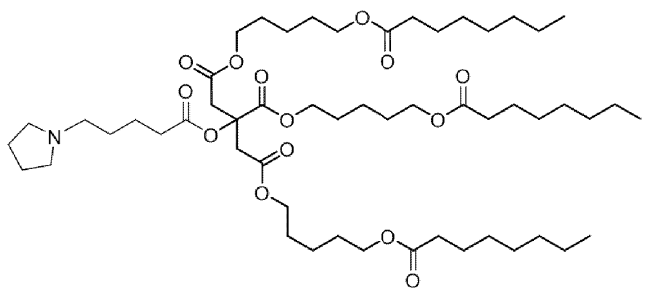
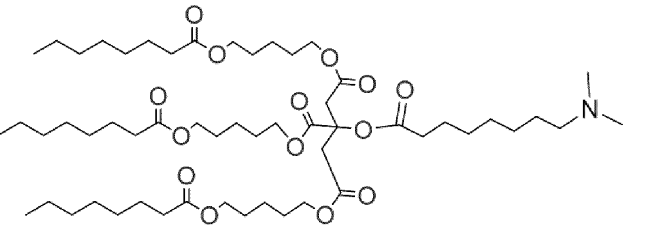
No.	Structure
102	
103	
104	
105	
106	

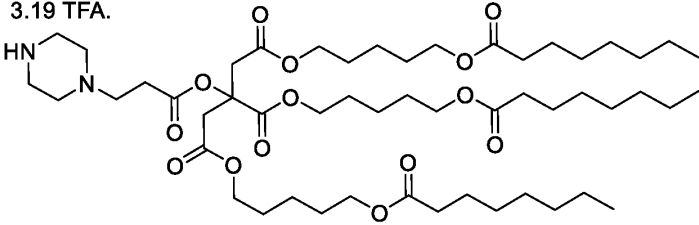
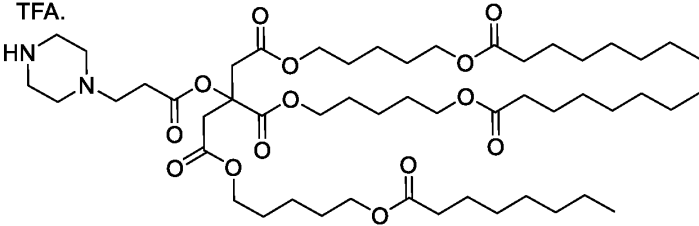
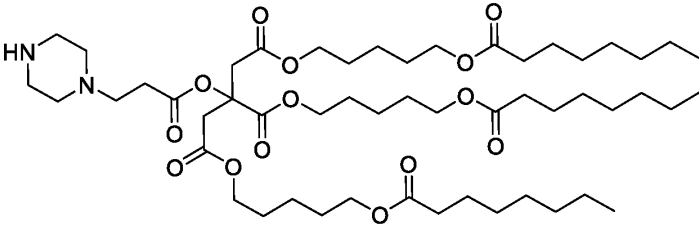
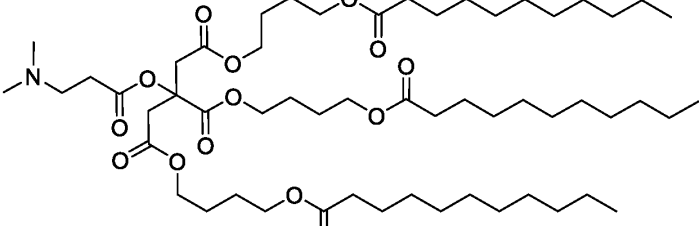
No.	Structure
107	
108	
109	1.62 TFA TFA content 15% (w/w)
110	TFA

No.	Structure
111	

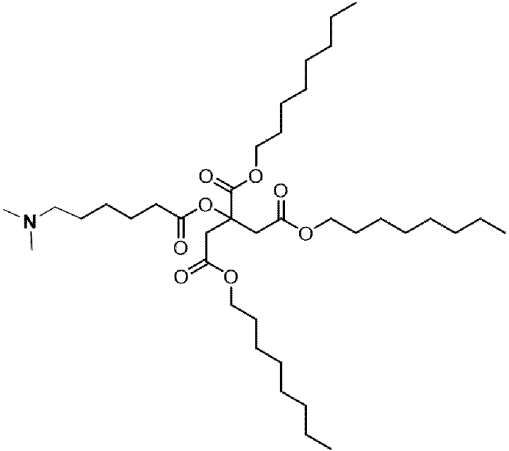
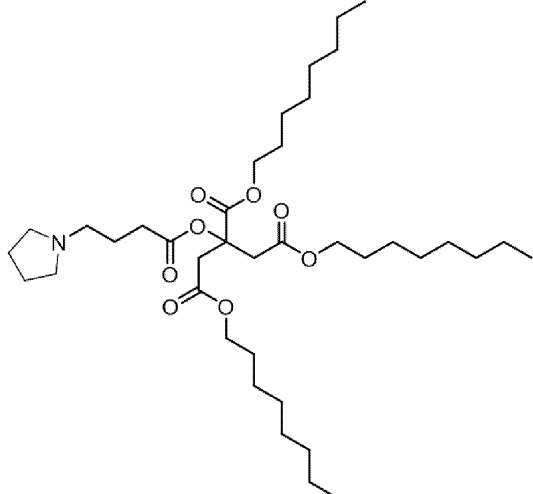
(c) Compounds 112-117 or 126-129, or a pharmaceutically acceptable salt thereof:

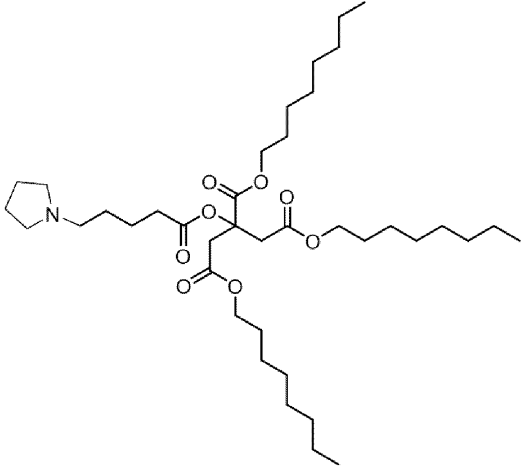
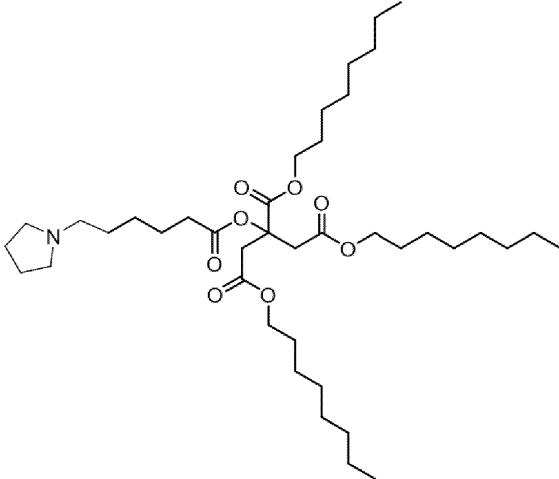
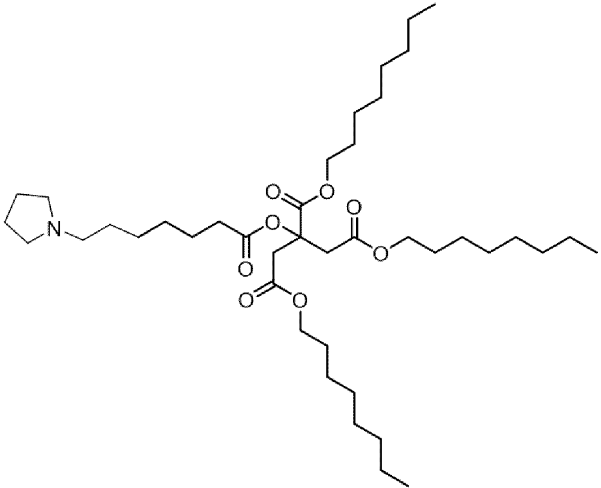
No.	Structure
112	
113	

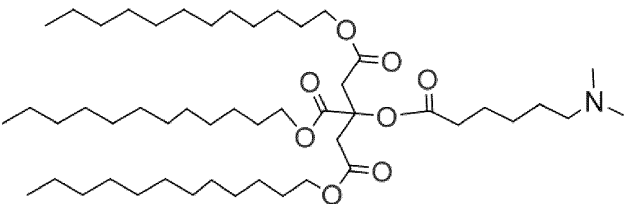
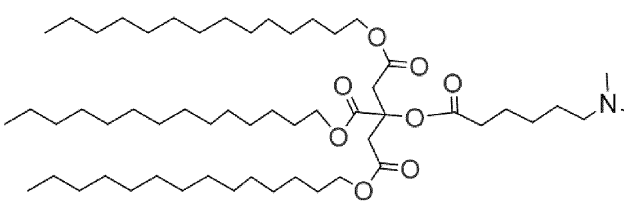
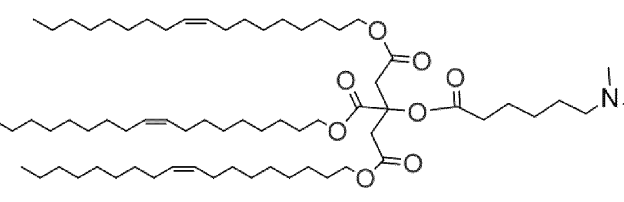
No.	Structure
114	
115	
116	
117	

No.	Structure
126	3.19 TFA. 
127	TFA. 
128	
129	

(d) Compounds 118-125, or a pharmaceutically acceptable salt thereof:

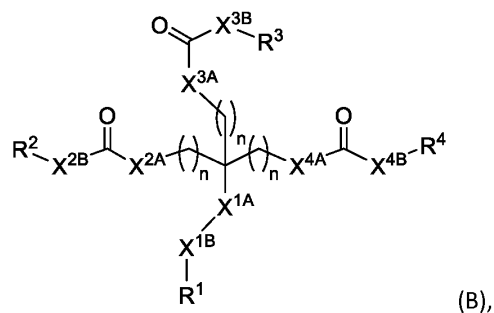
118	
119	

120	 <chem>CCCCCCCCCOC(=O)C(C(=O)OCCCCCCCCC)(C(=O)OCCCCCCCCC)C(=O)OCCCCN1CCCC1</chem>
121	 <chem>CCCCCCCCCOC(=O)C(C(=O)OCCCCCCCCC)(C(=O)OCCCCCCCCC)C(=O)OCCCCN1CCCC1</chem>
122	 <chem>CCCCCCCCCOC(=O)C(C(=O)OCCCCCCCCC)(C(=O)OCCCCCCCCC)C(=O)OCCCCN1CCCC1</chem>

123	
124	
125	

9. A composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound of any one of claims 1–8.
10. A method of pulmonary, intranasal, or intramuscular delivery of a composition comprising an mRNA encoding a protein or polypeptide, encapsulated within a liposome, wherein the liposome comprises one or more cationic lipids, optionally one or more non-cationic lipids, optionally one or more cholesterol-based lipids, and optionally one or more PEG-

modified lipids, wherein at least one cationic lipid is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A} ;

R^{1A} is H or C_1 - C_6 alkyl;

X^{1B} is a covalent bond, $C(O)$, CH_2CO_2 , or $CH_2C(O)$;

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R^1 is independently L^1 - B^1 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^2 is independently L^2 - B^2 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^3 is independently L^3 - B^3 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

R^4 is independently L^4 - B^4 , C_6 - C_{30} alkyl, C_6 - C_{30} alkenyl, or C_6 - C_{30} alkynyl;

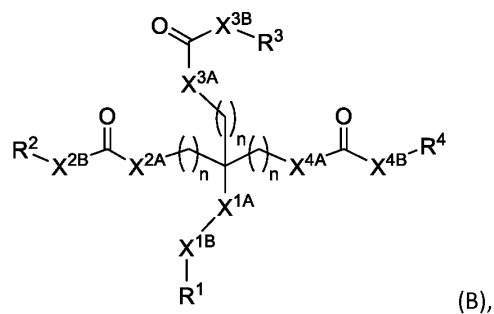
L^1 , L^2 , L^3 , and L^4 are each independently C_1 - C_{30} alkylene; C_2 - C_{30} alkenylene; or C_2 - C_{30} alkynylene;

each of B^1 , B^2 , B^3 , and B^4 is independently an ionizable nitrogen-containing group,

and

wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

11. A method of pulmonary, intranasal, or intramuscular delivery of a composition comprising a nucleic acid encapsulated within a liposome, wherein the liposome comprises a cationic lipid that is a compound having a structure according to Formula (B):



or a pharmaceutically acceptable salt thereof, wherein

each n is independently 0 or 1;

X^{1A} is independently O or NR^{1A};

R^{1A} is H or C₁-C₆ alkyl;

X^{1B} is a covalent bond, C(O), CH₂CO₂, or CH₂C(O);

one of X^{2A} and X^{2B} is O and the other is a covalent bond;

one of X^{3A} and X^{3B} is O and the other is a covalent bond;

one of X^{4A} and X^{4B} is O and the other is a covalent bond;

R¹ is independently L¹-B¹, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R² is independently L²-B², C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R³ is independently L³-B³, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

R⁴ is independently L⁴-B⁴, C₆-C₃₀ alkyl, C₆-C₃₀ alkenyl, or C₆-C₃₀ alkynyl;

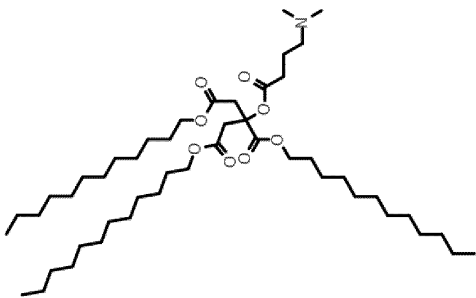
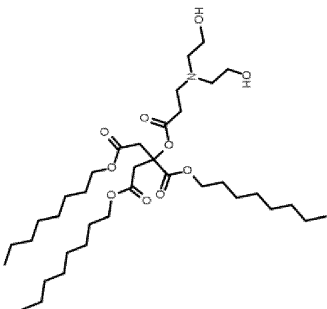
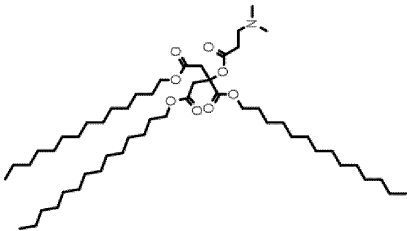
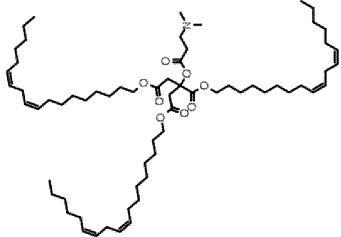
L¹, L², L³, and L⁴ are each independently C₁-C₃₀ alkylene; C₂-C₃₀ alkenylene; or C₂-C₃₀ alkynylene;

each of B¹, B², B³, and B⁴ is independently an ionizable nitrogen-containing group,

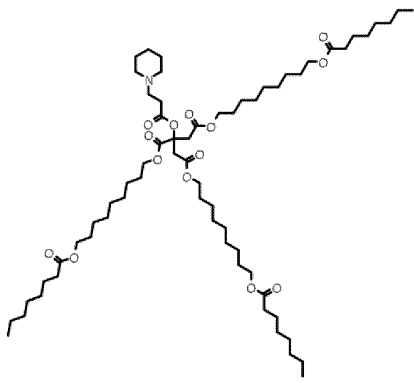
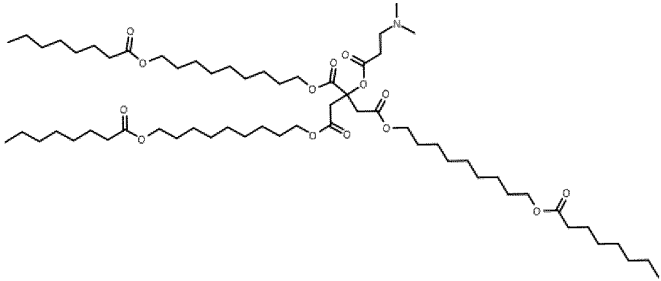
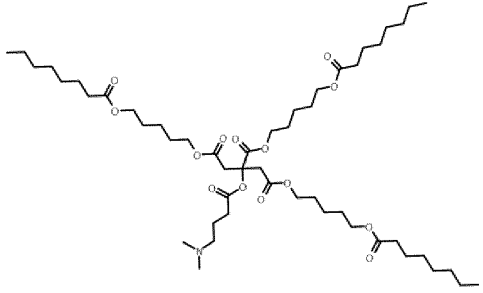
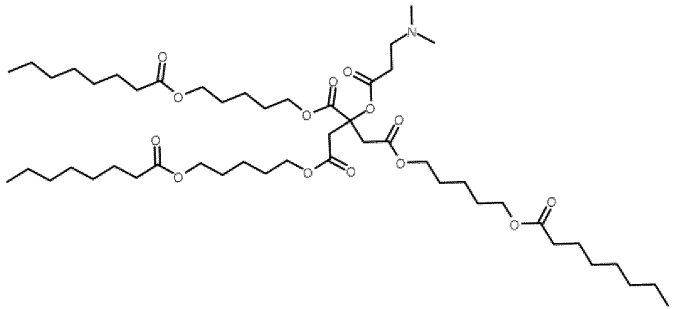
and

wherein the cationic lipid comprises at least one ionizable nitrogen-containing group.

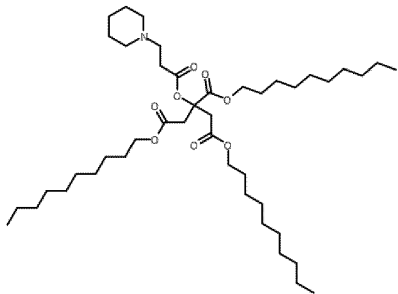
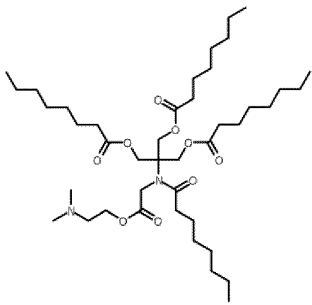
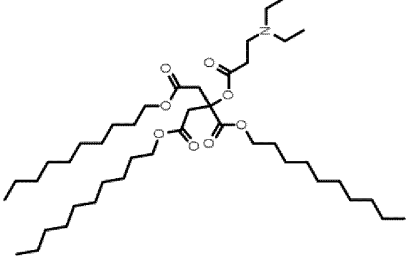
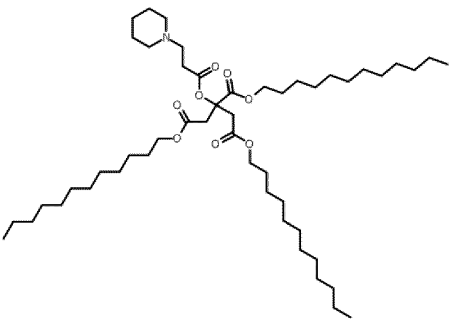
12. The method of claim 10 or 11, wherein the compound is selected from the group consisting of (a) Compounds (65)-(89), or a pharmaceutically acceptable salt thereof:

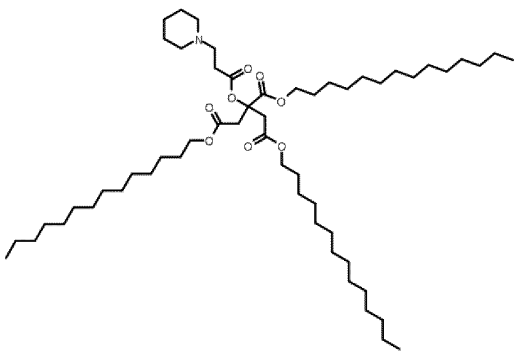
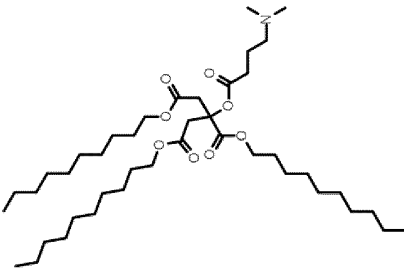
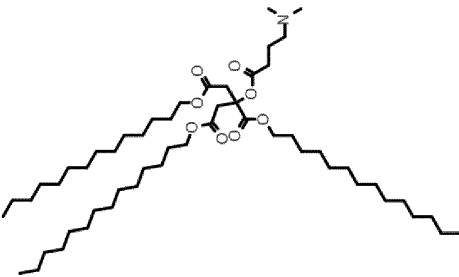
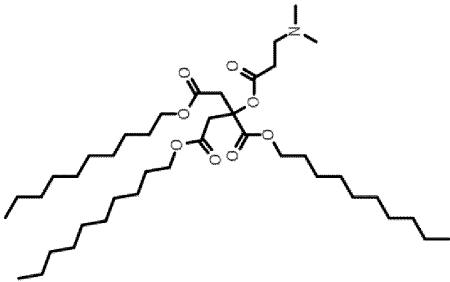
No.	Structure
65	
66	
67	
68	

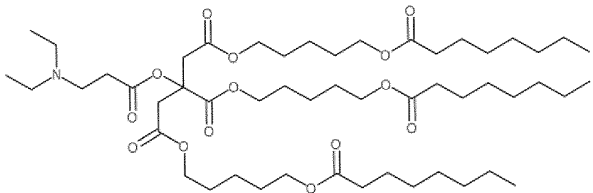
No.	Structure
69	
70	
71	
72	

No.	Structure
73	
74	
75	
76	

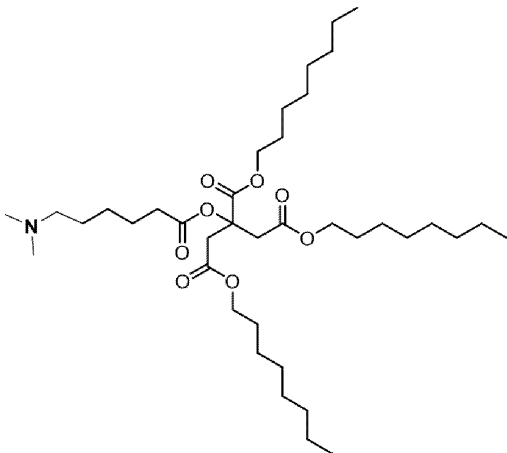
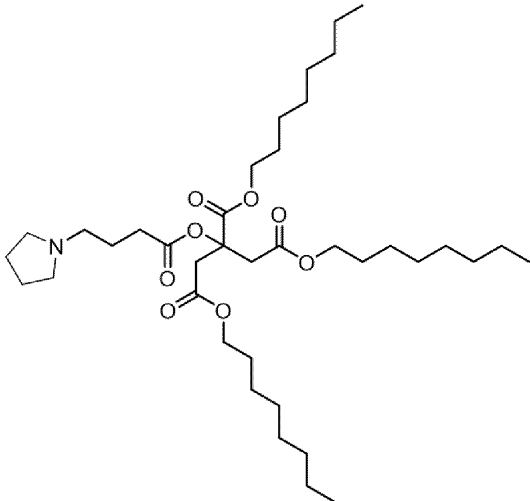
No.	Structure
77	
78	
79	
80	

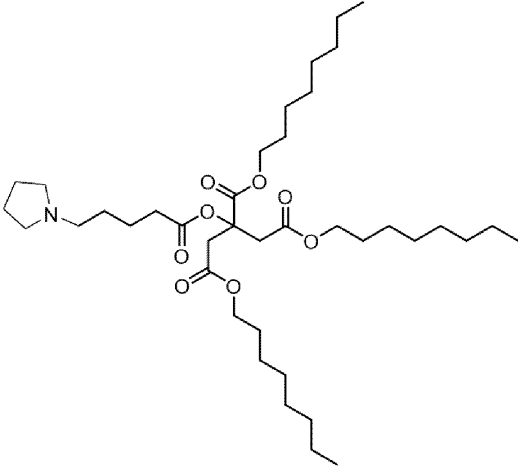
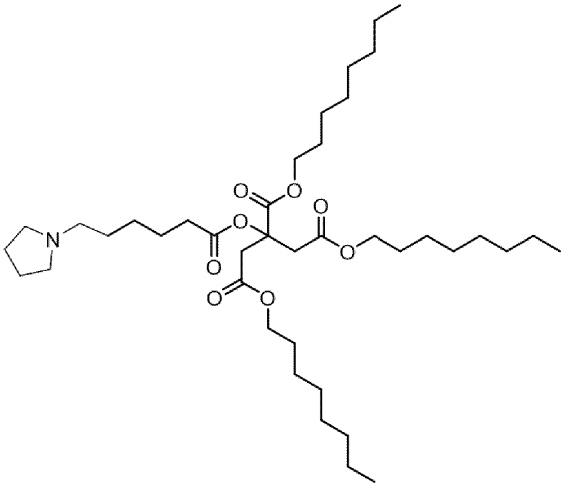
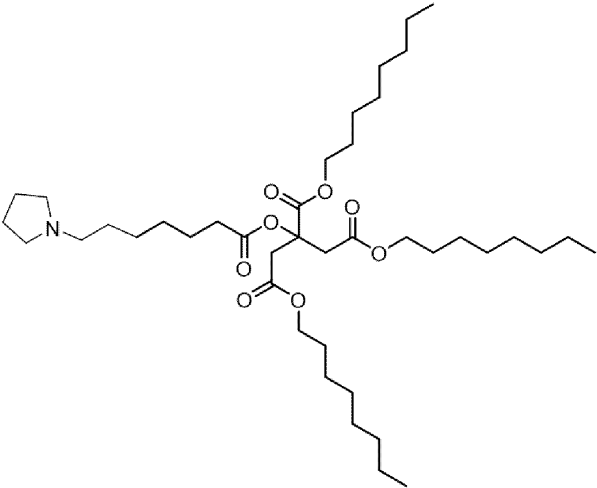
No.	Structure
81	
82	
83	
84	

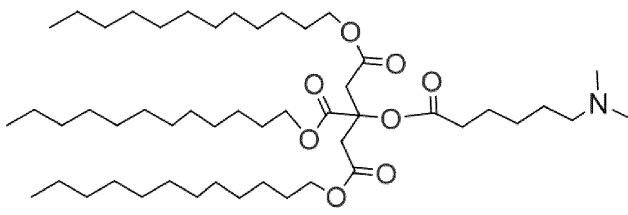
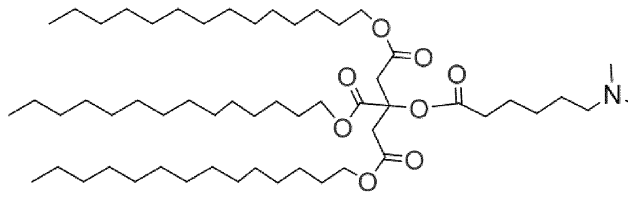
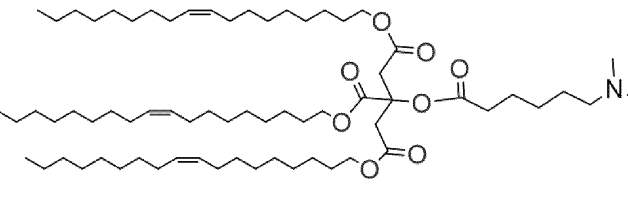
No.	Structure
85	
86	
87	
88	

No.	Structure
89	

(b) Compounds 118-125, or a pharmaceutically acceptable salt thereof:

118	
119	

120	
121	
122	

123	
124	
125	

13. The method of any one of claims 10-12, wherein said delivery is intranasal.
14. The method of any one of claims 10-12, wherein said delivery is intramuscular.
15. The method of any one of claims 10-12, wherein said delivery is pulmonary.