(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 June 2013 (13.06.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/086354 A1

(51) International Patent Classification:
A61K 31/713 (2006.01) *C07C 211/10* (2006.01)
A61K 31/7088 (2006.01) *C07C 211/11* (2006.01)
C07F 5/02 (2006.01) *C07C 217/08* (2006.01)
C07C 229/12 (2006.01) *C07C 251/38* (2006.01)
C07C 327/22 (2006.01) *C07C 327/28* (2006.01)
C07C 327/32 (2006.01) *C07D 317/30* (2006.01)
C07C 211/09 (2006.01) *C07D 235/06* (2006.01)

(21) International Application Number:
PCT/US2012/068491

(22) International Filing Date:
7 December 2012 (07.12.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/568,133 7 December 2011 (07.12.2011) US
61/623,274 12 April 2012 (12.04.2012) US

(71) Applicant: **ALNYLAM PHARMACEUTICALS, INC.** [US/US]; 300 Third Street, Cambridge, MA 02142 (US).

(72) Inventors: **MAIER, Martin**; 300 Third Street, Cambridge, MA 02142 (US). **JAYARAMAN, Muthusamy**; 300 Third Street, Cambridge, MA 02142 (US). **AKINC, Akin**; 300 Third Street, Cambridge, MA 02142 (US). **MATSUDA, Shigeo**; 300 Third Street, Cambridge, MA 02142 (US). **KADASAMY, Pachamuthu**; 99 Florence Street, Malden, MA 02148 (US). **RAJEEV, Kallanthottathil, G.**; 300 Third Street, Cambridge, MA 02142 (US). **MANOHARAN, Muthiah**; 300 Third Street, Cambridge, MA 02142 (US).

(74) Agent: **LESSLER, Jay, P.**; Blank Rome LLP, The Chrysler Building, 405 Lexington Ave., New York, NY 10174-0208 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2013/086354 A1

(54) **Title**: BIODEGRADABLE LIPIDS FOR THE DELIVERY OF ACTIVE AGENTS

(57) **Abstract**: The present invention relates to a cationic lipid having one or more biodegradable groups located in a lipidic moiety (e.g., a hydrophobic chain) of the cationic lipid. These cationic lipids may be incorporated into a lipid particle for delivering an active agent, such as a nucleic acid. The invention also relates to lipid particles comprising a neutral lipid, a lipid capable of reducing aggregation, a cationic lipid of the present invention, and optionally, a sterol. The lipid particle may further include a therapeutic agent such as a nucleic acid.

BIODEGRADABLE LIPIDS FOR THE DELIVERY OF ACTIVE AGENTS

This application claims the benefit of U.S. Provisional Application No. 61/568,133, filed December 7, 2011, and U.S. Provisional Application No. 61/623,274, filed April 12, 2012, both of which are hereby incorporated by reference.

Technical Field

The present invention relates to biodegradable lipids and to their use for the delivery of active agents such as nucleic acids.

Background

Therapeutic nucleic acids include, *e.g.*, small interfering RNA (siRNA), micro RNA (miRNA), antisense oligonucleotides, ribozymes, plasmids, immune stimulating nucleic acids, antisense, antagomir, antimir, microRNA mimic, supermir, U1 adaptor, and aptamer. In the case of siRNA or miRNA, these nucleic acids can down-regulate intracellular levels of specific proteins through a process termed RNA interference (RNAi). The therapeutic applications of RNAi are extremely broad, since siRNA and miRNA constructs can be synthesized with any nucleotide sequence directed against a target protein. To date, siRNA constructs have shown the ability to specifically down-regulate target proteins in both *in vitro* and *in vivo* models. In addition, siRNA constructs are currently being evaluated in clinical studies.

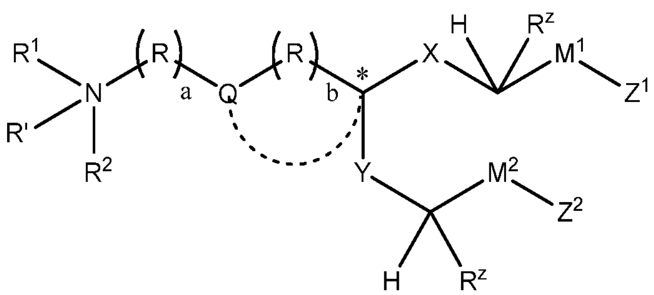
However, two problems currently faced by siRNA or miRNA constructs are, first, their susceptibility to nuclease digestion in plasma and, second, their limited ability to gain access to the intracellular compartment where they can bind the protein RISC when administered systemically as the free siRNA or miRNA. Lipid nanoparticles formed from cationic lipids with other lipid components, such as cholesterol and PEG lipids, and oligonucleotides (such as siRNA and miRNA) have been used to facilitate the cellular uptake of the oligonucleotides.

There remains a need for improved cationic lipids and lipid nanoparticles for the delivery of oligonucleotides. Preferably, these lipid nanoparticles would provide high drug:lipid ratios, protect the nucleic acid from degradation and clearance in serum, be suitable for systemic delivery, and provide intracellular delivery of the nucleic acid. In addition, these lipid-nucleic acid particles should be well-tolerated and provide an adequate therapeutic index, such that patient treatment at an effective dose of the nucleic acid is not associated with significant toxicity and/or risk to the patient.

Summary

The present invention relates to a cationic lipid and PEG lipid suitable for forming nucleic acid-lipid particles. Each of the cationic and PEG lipids of the present invention includes one or more biodegradable groups. The biodegradable groups are located in a lipidic moiety (e.g., a hydrophobic chain) of the cationic or PEG lipid. These cationic and PEG lipids may be incorporated into a lipid particle for delivering an active agent, such as a nucleic acid (e.g., an siRNA). The incorporation of the biodegradable group(s) into the lipid results in faster metabolism and removal of the lipid from the body following delivery of the active agent to a target area. As a result, these lipids have lower toxicity than similar lipids without the biodegradable groups.

In one embodiment, the cationic lipid is a compound of formula (I), which has a branched alkyl at the alpha position adjacent to the biodegradable group (between the biodegradable group and the teriary carbon):



Formula (I)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R’ is absent, hydrogen, or alkyl (e.g., C_1-C_4 alkyl);

with respect to R^1 and R^2,

(i) R^1 and R^2 are each, independently, optionally substituted alkyl, alkenyl, alkynyl, cycloalkylalkyl, heterocycle, or R^{10};

(ii) R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring; or

(iii) one of R^1 and R^2 is optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, or heterocycle, and the other forms a 4-10 member heterocyclic ring or heteroaryl (e.g., a 6-member ring) with (a) the adjacent nitrogen atom and (b) the $(R)_a$ group adjacent to the nitrogen atom;

each occurrence of R is, independently, $-(CR^3R^4)-$;

each occurrence of R^3 and R^4 are, independently H, halogen, OH, alkyl, alkoxy, $-NH_2$, R^{10}, alkylamino, or dialkylamino (in one preferred embodiment, each occurrence of R^3 and R^4 are, independently H or C_1-C_4 alkyl);

each occurrence of R^{10} is independently selected from PEG and polymers based on poly(oxazoline), poly(ethylene oxide), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), poly[N-(2-hydroxypropyl)methacrylamide] and poly(amino acid)s, wherein (i) the PEG or polymer is linear or branched, (ii) the PEG or polymer is polymerized by n subunits, (iii) n is a number-averaged degree of polymerization between 10 and 200 units, and (iv) wherein the compound of formula has at most two R^{10} groups (preferably at most one R^{10} group);

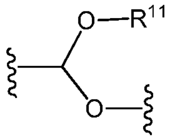
the dashed line to Q is absent or a bond;

when the dashed line to Q is absent then Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)N(R^4)-, -N(R^5)C(O)-, -S-S-, -OC(O)O-, -O-N=C(R^5)-, -C(R^5)=N-O-, -OC(O)N(R^5)-, -N(R^5)C(O)N(R^5)-, -N(R^5)C(O)O-, -C(O)S-, -C(S)O- or -C(R^5)=N-O-C(O)-; or

when the dashed line to Q is a bond then (i) b is 0 and (ii) Q and the tertiary carbon adjacent to it (C*) form a substituted or unsubstituted, mono- or bi-cyclic heterocyclic group having from 5 to 10 ring atoms (e.g., the heteroatoms in the heterocyclic group are selected from O and S, preferably O);

each occurrence of R^5 is, independently, H or alkyl (e.g. C_1-C_4 alkyl);

X and Y are each, independently, alkylene or alkenylene (e.g., C_4 to C_{20} alkylene or C_4 to C_{20} alkenylene);

M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or 

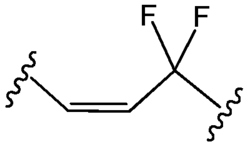
(wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

each occurrence of R^z is, independently, C_1-C_8 alkyl (e.g., methyl, ethyl, isopropyl, n-butyl, n-pentyl, or n-hexyl);

a is 1, 2, 3, 4, 5 or 6;

b is 0, 1, 2, or 3; and

Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2 (e.g.,



).

The $R'R^1R^2N-(R)_a-Q-(R)_b-$ group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, $R'R^1R^2N-(R)_a-Q-(R)_b-$ is $(CH_3)_2N-(CH_2)_3-C(O)O-$, $(CH_3)_2N-(CH_2)_2-NH-C(O)O-$, $(CH_3)_2N-(CH_2)_2-OC(O)-NH-$, or $(CH_3)_2N-(CH_2)_3-C(CH_3)=N-O-$.

In one embodiment, R^1 and R^2 are both alkyl (e.g., methyl).

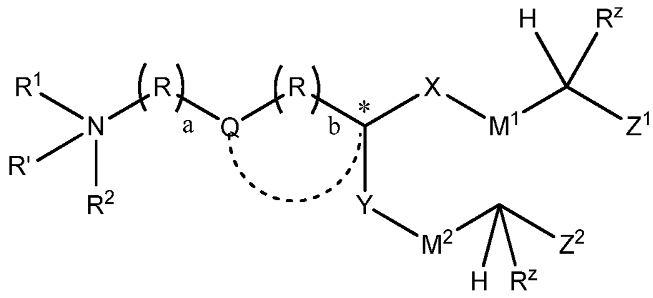
In a further embodiment, a is 3. In another embodiment, b is 0.

In a further embodiment, a is 3, b is 0 and R is $-CH_2-$. In yet a further embodiment, a is 3, b is 0, R is $-CH_2-$ and Q is $-C(O)O-$. In another embodiment, R^1 and R^2 are methyl, a is 3, b is 0, R is $-CH_2-$ and Q is $-C(O)O-$.

In another embodiment, X and Y are each, independently $-(CH_2)_n-$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are $-(CH_2)_6-$. In another embodiment, X and Y are $-(CH_2)_7-$. In yet another embodiment, X and Y are $-(CH_2)_9-$. In yet another embodiment, X and Y are $-(CH_2)_8-$.

In further embodiments, M^1 and M^2 are each, independently, -OC(O)- or -C(O)O-. For example, in one embodiment, M^1 and M^2 are each $-C(O)O-$.

In another embodiment, the cationic lipid is a compound of formula (II), which has a branched alkyl at the alpha position adjacent to the biodegradable group (between the biodegradable group and the terminus of the tail, i.e., Z^1 o Z^2):



Formula (II)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R' is absent, hydrogen, or alkyl (e.g., C_1-C_4 alkyl);

with respect to R^1 and R^2,

(i) R^1 and R^2 are each, independently, optionally substituted alkyl, alkenyl, alkynyl, cycloalkylalkyl, heterocycle, or R^{10};

(ii) R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring; or

(iii) one of R^1 and R^2 is optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, or heterocycle, and the other forms a 4-10 member heterocyclic ring or heteroaryl (e.g., a 6-member ring) with (a) the adjacent nitrogen atom and (b) the $(R)_a$ group adjacent to the nitrogen atom;

each occurrence of R is, independently, $-(CR^3R^4)-$;

each occurrence of R^3 and R^4 are, independently H, halogen, OH, alkyl, alkoxy, $-NH_2$, R^{10}, alkylamino, or dialkylamino (in one preferred embodiment, each occurrence of R^3 and R^4 are, independently H or C_1-C_4 alkyl);

each occurrence of R^{10} is independently selected from PEG and polymers based on poly(oxazoline), poly(ethylene oxide), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), poly[N-(2-hydroxypropyl)methacrylamide] and poly(amino acid)s, wherein

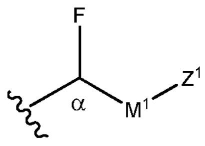
(i) the PEG or polymer is linear or branched, (ii) the PEG or polymer is polymerized by n subunits, (iii) n is a number-averaged degree of polymerization between 10 and 200 units, and (iv) wherein the compound of formula has at most two R^{10} groups (preferably at most one R^{10} group);

the dashed line to Q is absent or a bond;

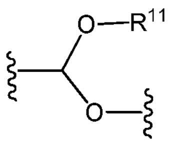
when the dashed line to Q is absent then Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)N(R^4)-, -N(R^5)C(O)-, -S-S-, -OC(O)O-, -O-N=C(R^5)-, -C(R^5)=N-O-, -OC(O)N(R^5)-, -N(R^5)C(O)N(R^5)-, -N(R^5)C(O)O-, -C(O)S-, -C(S)O- or -C(R^5)=N-O-C(O)-; or

when the dashed line to Q is a bond then (i) b is 0 and (ii) Q and the tertiary carbon adjacent to it (C*) form a substituted or unsubstituted, mono- or bi-cyclic heterocyclic group having from 5 to 10 ring atoms (e.g., the heteroatoms in the heterocyclic group are selected from O and S, preferably O);

each occurrence of R^5 is, independently, H or alkyl;

X and Y are each, independently, alkylene (e.g., C_6-C_8 alkylene) or alkenylene, wherein the alkylene or alkenylene group is optionally substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., 

);

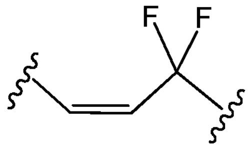
M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or 

(wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

each occurrence of R^z is, independently, C_1-C_8 alkyl (e.g., methyl, ethyl, isopropyl);

a is 1, 2, 3, 4, 5 or 6;

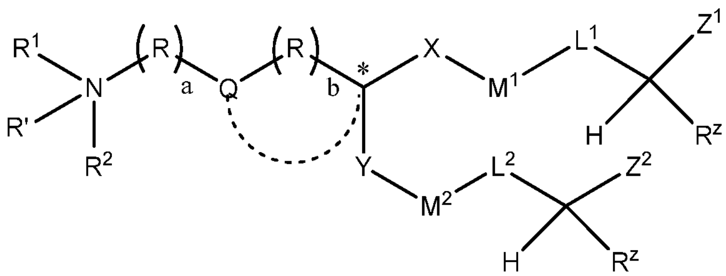
b is 0, 1, 2, or 3; and

Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein (i) the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2 (e.g., ) and (ii) the terminus of at least one of Z^1 and Z^2 is separated from the group M^1 or M^2 by at least 8 carbon atoms.

In another embodiment, X and Y are each, independently $-(CH_2)_n-$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are $-(CH_2)_6-$. In another embodiment, X and Y are $-(CH_2)_7-$. In yet another embodiment, X and Y are $-(CH_2)_9-$. In yet another embodiment, X and Y are $-(CH_2)_8-$.

The $R'R^1R^2N-(R)_a-Q-(R)_b-$ group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, $R'R^1R^2N-(R)_a-Q-(R)_b-$ is $(CH_3)_2N-(CH_2)_3-C(O)O-$, $(CH_3)_2N-(CH_2)_2-NH-C(O)O-$, $(CH_3)_2N-(CH_2)_2-OC(O)-NH-$, or $(CH_3)_2N-(CH_2)_3-C(CH_3)=N-O-$.

In another embodiment, the cationic lipid is a compound of formula (III), which has a branching point at a position that is 2-6 carbon atoms (i.e., at the beta (β), gamma (γ), delta (δ), epsilon (ε) or zeta position(ζ)) adjacent to the biodegradable group (between the biodegradable group and the terminus of the tail, i.e., Z^1 or Z^2):



Formula (III)

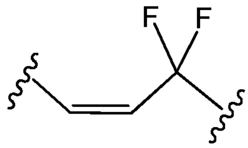
or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R', R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, M^1, M^2, R^z, a, and b are defined as in formula (I);

L^1 and L^2 are each, independently, C_1-C_5 alkylene or C_2-C_5 alkenylene;

X and Y are each, independently, alkylene (e.g., C_4 to C_{20} alkylene or C_6-C_8 alkylene) or alkenylene (e.g., C_4 to C_{20} alkenylene); and

Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2 (e.g.,



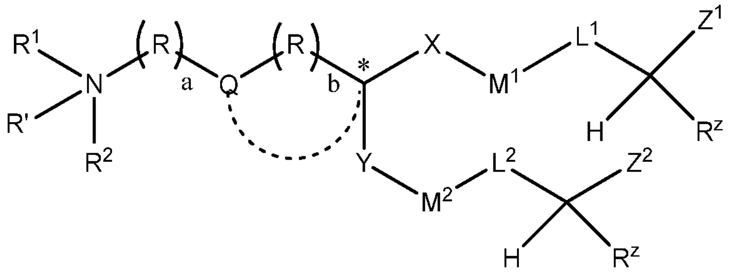
), and with the proviso that the terminus of at least one of Z^1 and Z^2 is separated from the group M^1 or M^2 by at least 8 carbon atoms.

In one embodiment, L^1 and L^2 are each -CH_2-. In another embodiment, L^1 and L^2 are each -$(CH_2)_2$-. In one embodiment, L^1 and L^2 are each -$(CH_2)_3$-. In yet another embodiment, L^1 and L^2 are each -$(CH_2)_4$-. In yet another embodiment, L^1 and L^2 are each -$(CH_2)_5$-. In yet another embodiment, L^1 and L^2 are each –CH_2-CH=CH-. In a preferred embodiment, L^1 and L^2 are each -CH_2- or -$(CH_2)_2$.

In one embodiment, X and Y are each, independently –$(CH_2)_n$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are -$(CH_2)_7$-. In another exemplary embodiment, X and Y are -$(CH_2)_8$-. In yet another exemplary embodiment, X and Y are -$(CH_2)_9$-.

The R'R^1R^2N-$(R)_a$-Q-$(R)_b$- group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, R'R^1R^2N-$(R)_a$-Q-$(R)_b$- is $(CH_3)_2$N-$(CH_2)_3$-C(O)O-, $(CH_3)_2$N-$(CH_2)_2$-NH-C(O)O-, $(CH_3)_2$N-$(CH_2)_2$-OC(O)-NH-, or $(CH_3)_2$N-$(CH_2)_3$-C(CH_3)=N-O-.

In another embodiment, the cationic lipid is a compound of formula (IIIA), which has a branching point at a position that is 2-6 carbon atoms (i.e., at the beta (β), gamma (γ), delta (δ), epsilon (ε) or zeta position(ζ)) from the biodegradable groups M^1 and M^2 (i.e., between the biodegradable group and the terminus of the tail, i.e., Z^1 or Z^2):



Formula (IIIA)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R’, R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, M^1, M^2, a, and b are defined as in formula (I);

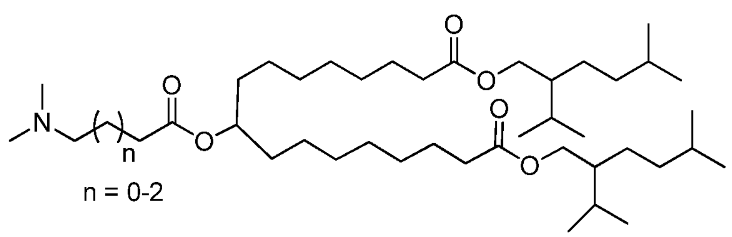
each R^z is, independently, C_1-C_8 alkyl (e.g., C_3-C_6 alkyl or C_2-C_3 alkyl);

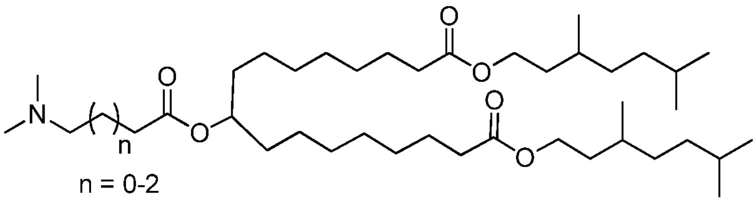
L^1 and L^2 are each, independently, C_1-C_5 alkylene (e.g., C_2-C_3 alkylene) or C_2-C_5 alkenylene;

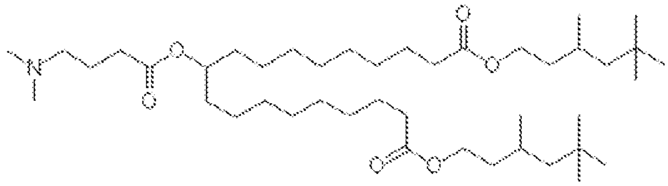
X and Y are each, independently, alkylene (e.g., C_4 to C_{20} alkylene or C_7-C_9 alkylene) or alkenylene (e.g., C_4 to C_{20} alkenylene or C_7-C_9 alkenylene); and

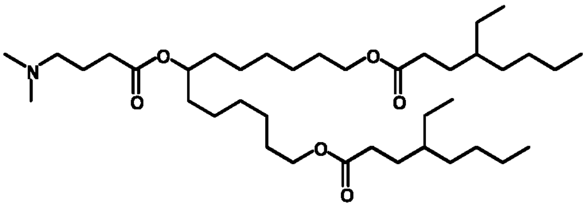
Z^1 and Z^2 are each, independently, C_1-C_8 alkyl (e.g., C_1-C_6 alkyl, such as C_1, C_3 or C_5 alkyl) or C_2-C_8 alkenyl (such as C_2-C_6 alkenyl);

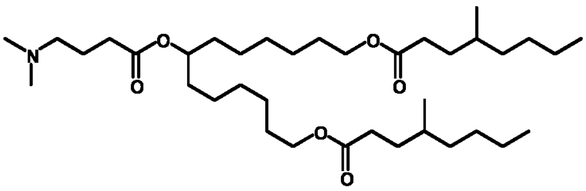
wherein said cationic lipid is not one selected from:

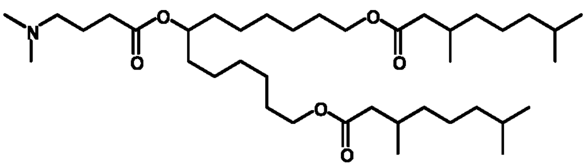


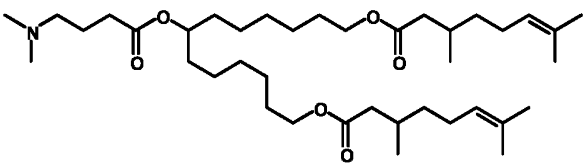


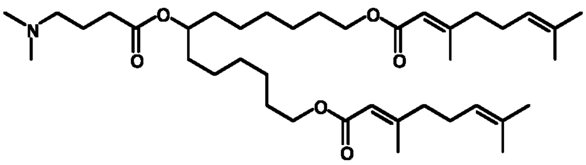












In one embodiment, L^1 and L^2 are each $-(CH_2)_2-$. In another embodiment, L^1 and L^2 are each $-(CH_2)_3-$.

In one embodiment, X and Y are each, independently $-(CH_2)_n$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, 4 to 12 or 7-9. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are $-(CH_2)_7-$. In yet another exemplary embodiment, X and Y are $-(CH_2)_9$.

In one preferred embodiment, M^1 and M^2 are –C(O)O- (where the carbonyl group in M^1 and M^2 is bound to the variable X, and the oxygen atom in M^1 and M^2 is bound to the variable L^1 and L^2).

The $R'R^1R^2N-(R)_a-Q-(R)_b-$ group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, $R'R^1R^2N-(R)_a-Q-(R)_b-$ is $(CH_3)_2N-(CH_2)_3-C(O)O-$, $(CH_3)_2N-(CH_2)_2-NH-C(O)O-$, $(CH_3)_2N-(CH_2)_2-OC(O)-NH-$, or $(CH_3)_2N-(CH_2)_3-C(CH_3)=N-O-$.

In one preferred embodiment, Z^1 and Z^2 are branched alkyl or branched alkenyl groups.

In one embodiment of formula (IIIA), Z^1, Z^2, and each R^z are C_3-C_8 alkyl (such as a C_3-C_6 alkyl). In another embodiment of formula (IIIA), Z^1, Z^2, and each R^z are C_3-C_8 branched alkyl (such as a C_3-C_6 branched alkyl). In yet another embodiment of formula (IIIA), Z^1, Z^2, and each R^z are C_3-C_8 straight alkyl (such as a C_3-C_6 straight alkyl).

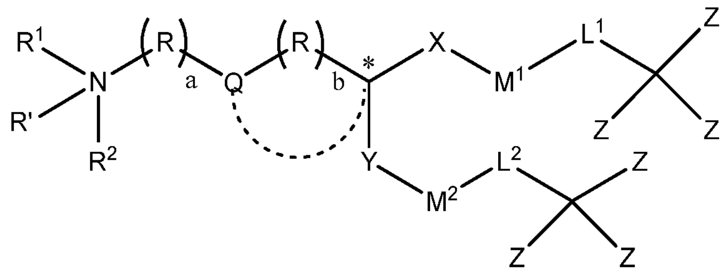
In one embodiment of formula (IIIA), the branching point is at the second position (the β-position) from the biodegradable groups M^1 and M^2 in each tail. Z^1, Z^2, and each R^z can be C_3-C_8 alkyl (e.g., a C_3-C_6 alkyl), such as a C_3-C_8 branched alkyl (e.g., a C_3-C_6 branched alkyl) or a C_3-C_8 straight alkyl (e.g., a C_3-C_6 straight alkyl). In one preferred embodiment, M^1 and M^2 are –C(O)O- (where the carbonyl group in M^1 and M^2 is bound to the variable X, and the oxygen atom in M^1 and M^2 is bound to the variable L^1 and/or L^2).

In one embodiment of formula (IIIA), the branching point is at the third position (the γ-position) from the biodegradable groups M^1 and M^2 in each tail. Z^1, Z^2, and each R^z can be C_3-C_8 alkyl (e.g., a C_3-C_6 alkyl), such as a C_3-C_8 branched alkyl (e.g., a C_3-C_6 branched alkyl) or a C_3-C_8 straight alkyl (e.g., a C_3-C_6 straight alkyl). In one preferred embodiment, M^1 and M^2 are –C(O)O- (where the carbonyl group in M^1 and M^2 is bound to the variable X, and the oxygen atom in M^1 and M^2 is bound to the variable L^1 and/or L^2).

In one embodiment of formula (IIIA), the branching point is at the third position (the γ-position) from the biodegradable groups M^1 and M^2 in each tail.

In another embodiment of formula (IIIA), M^1 and/or M^2 are not –O(C(O)- (where the oxygen atom in M^1 and/or M^2 is bound to the variable X, and the carbonyl in M^1 and/or M^2 is bound to the variable L^1 and/or L^2). In yet another embodiment of formula (IIIA), Z^1, Z^2, and R^z are not C_3-C_{10} cycloalkyl(C_1-C_6 alkyl).

In another embodiment, the cationic lipid is a compound of formula (IV), which has a branching point at a position that is 2-6 carbon atoms (i.e., at beta (β), gamma (γ), delta (δ), epsilon (ε) or zeta position(ζ)) adjacent to the biodegradable group (between the biodegradable group and the terminus of the tail, i.e., Z^1 or Z^2):



Formula (IV)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R', R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, M^1, M^2, R^z, a, and b are defined as in formula (I);

L^1 and L^2 and are each, independently, C_1-C_5 alkylene or C_2-C_5 alkenylene;

X and Y are each, independently, alkylene or alkenylene (e.g., C_{12}-C_{20} alkylene or C_{12}-C_{20} alkenylene); and

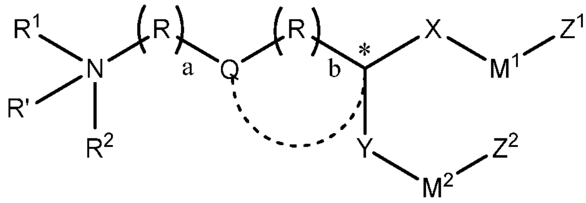
each occurrence of Z is independently C_1-C_4 alkyl (preferably, methyl).

For example, in one embodiment, -L^1-$C(Z)_3$ is –$CH_2C(CH_3)_3$. In another embodiment, -L^1-$C(Z)_3$ is –$CH_2CH_2C(CH_3)_3$.

In one embodiment, the total carbon atom content of each tail (e.g., -X-M^1-L^1-$C(Z)_3$ or –Y-M^2-L^2-$C(Z)_3$) is from about 17 to about 26. For example, the total carbon atom content can be from about 19 to about 26 or from about 21 to about 26.

In another embodiment, X and Y are each, independently –$(CH_2)_n$– wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are $-(CH_2)_6-$. In another embodiment, X and Y are $-(CH_2)_7-$. In yet another embodiment, X and Y are $-(CH_2)_9-$. In yet another embodiment, X and Y are $-(CH_2)_8-$.

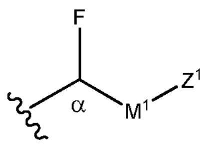
In one embodiment, the cationic lipid is a compound of formula (V), which has an alkoxy or thioalkoxy (i.e., -S-alkyl) group substitution on at least one tail:

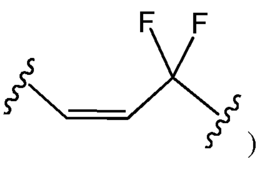


Formula (V)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R', R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, M^1, M^2, a, and b are defined as in formula (I);

X and Y are each, independently, alkylene (e.g., C_6-C_8 alkylene) or alkenylene, wherein the alkylene or alkenylene group is optionally substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., );

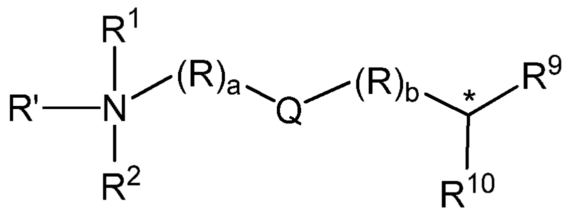
Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein (i) the C_8-C_{14} alkyl or C_8-C_{14} alkenyl of at least one of Z^1 and Z^2 is substituted by one or more alkoxy (e.g., a C_1-C_4 alkoxy such as $-OCH_3$) or thioalkoxy (e.g., a C_1-C_4 thioalkoxy such as $-SCH_3$) groups, and (ii) the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2 (e.g., ).

In one embodiment, the alkoxy substitution on Z^1 and/or Z^2 is at the beta position from the M^1 and/or M^2 group.

In another embodiment, X and Y are each, independently $-(CH_2)_n-$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are $-(CH_2)_6-$. In another embodiment, X and Y are $-(CH_2)_7-$. In yet another embodiment, X and Y are $-(CH_2)_9-$. In yet another embodiment, X and Y are $-(CH_2)_8-$.

The $R'R^1R^2N-(R)_a-Q-(R)_b-$ group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, $R'R^1R^2N-(R)_a-Q-(R)_b-$ is $(CH_3)_2N-(CH_2)_3-C(O)O-$, $(CH_3)_2N-(CH_2)_2-NH-C(O)O-$, $(CH_3)_2N-(CH_2)_2-OC(O)-NH-$, or $(CH_3)_2N-(CH_2)_3-C(CH_3)=N-O-$.

In one embodiment, the cationic lipid is a compound of formula (VIA), which has one or more fluoro substituents on at least one tail at a position that is either alpha to a double bond or alpha to a biodegradable group:



Formula (VIA)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R^1, R^2, R, a, and b are as defined with respect to formula (I);

Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, $-C(O)N(R^4)-$, $-N(R^5)C(O)-$, -S-S-, -OC(O)O-, $-O-N=C(R^5)-$, $-C(R^5)=N-O-$, $-OC(O)N(R^5)-$, $-N(R^5)C(O)N(R^5)-$, $-N(R^5)C(O)O-$, -C(O)S-, -C(S)O- or $-C(R^5)=N-O-C(O)-$;

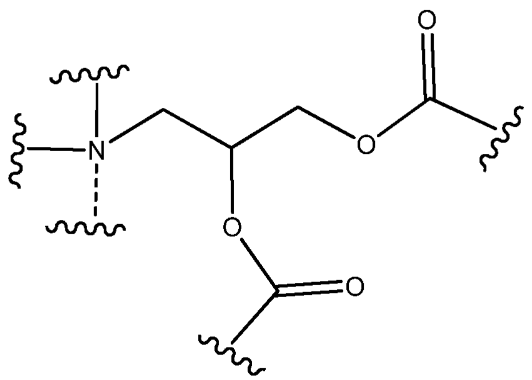
R' is absent, hydrogen, or alkyl (e.g., C_1-C_4 alkyl); and

each of R^9 and R^{10} are independently C_{12}-C_{24} alkyl (e.g., C_{12}-C_{20} alkyl), C_{12}-C_{24} alkenyl (e.g., C_{12}-C_{20} alkenyl), or C_{12}-C_{24} alkoxy (e.g., C_{12}-C_{20} alkoxy) (a) having one or more biodegradable groups and (b) optionally substituted with one or more fluorine atoms at a position which is (i) alpha to a biodegradable group and between the biodegradable group and the tertiary carbon atom marked with an asterisk (*), or (ii) alpha to a carbon-carbon double bond and between the double bond and the terminus of the R^9 or R^{10} group; each biodegradable group independently interrupts the C_{12}-C_{24} alkyl, alkenyl, or alkoxy group or is substituted at the terminus of the C_{12}-C_{24} alkyl, alkenyl, or alkoxy group,

wherein

(i) at least one of R^9 and R^{10} contains a fluoro group;

(ii) the compound does not contain the following moiety:



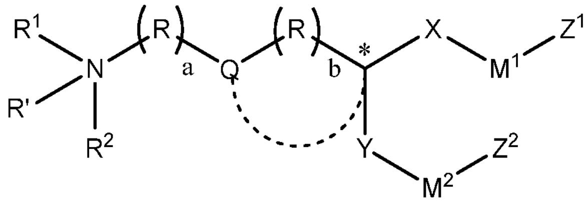
wherein ---- is an optional bond; and

(iii) the terminus of R^9 and R^{10} is separated from the tertiary carbon atom marked with an asterisk (*) by a chain of 8 or more atoms (e.g., 12 or 14 or more atoms).

In one preferred embodiment, the terminus of R^9 and R^{10} is separated from the tertiary carbon atom marked with an asterisk (*) by a chain of 18-22 carbon atoms (e.g., 18-20 carbon atoms).

In another embodiment, the terminus of the R^9 and/or R^{10} has the formula –C(O)O-CF_3.

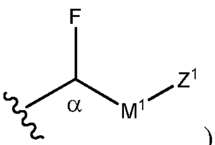
In another embodiment, the cationic lipid is a compound of formula (VIB), which has one or more fluoro substituents on at least one tail at a position that is either alpha to a double bond or alpha to a biodegradable group:



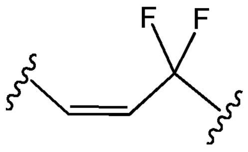
Formula (VIB)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

R', R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, M^1, M^2, a, and b are defined as in formula (I);

X and Y are each, independently, alkylene (e.g., C_6-C_8 alkylene) or alkenylene, wherein the alkylene or alkenylene group is optionally substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., 

); and

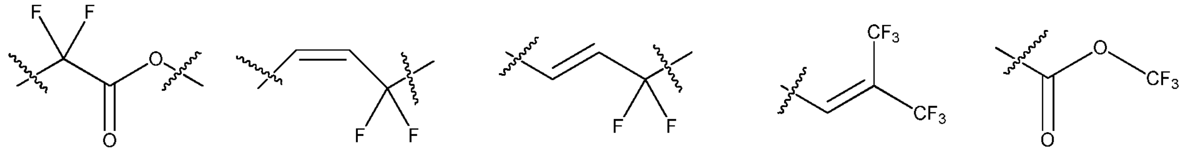
Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein said C_8-C_{14} alkenyl is optionally substituted by one or more fluorine atoms at a position that is alpha to a double bond (e.g., 

),

wherein at least one of X, Y, Z^1, and Z^2 contains a fluorine atom.

In one embodiment, at least one of Z^1 and Z^2 is substituted by two fluoro groups at a position that is either alpha to a double bond or alpha to a biodegradable group. In one embodiment, at least one of Z^1 and Z^2 has a terminal $-CF_3$ group at a position that is alpha to a biodegradable group (i.e., at least one of Z^1 and Z^2 terminates with an $-C(O)OCF_3$ group).

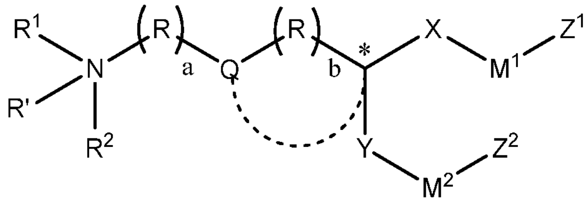
For example, at least one of Z^1 and Z^2 may include one or more of the following moieties:



In one embodiment, X and Y are each, independently $-(CH_2)_n$ wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are -$(CH_2)_7$-. In another exemplary embodiment, X and Y are -$(CH_2)_9$-. In yet another embodiment, X and Y are -$(CH_2)_8$-.

The R'R^1R^2N-$(R)_a$-Q-$(R)_b$- group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, R'R^1R^2N-$(R)_a$-Q-$(R)_b$- is $(CH_3)_2$N-$(CH_2)_3$-C(O)O-, $(CH_3)_2$N-$(CH_2)_2$-NH-C(O)O-, $(CH_3)_2$N-$(CH_2)_2$-OC(O)-NH-, or $(CH_3)_2$N-$(CH_2)_3$-C(CH_3)=N-O-.

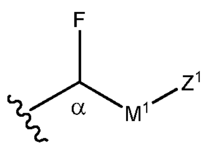
In one embodiment, the cationic lipid is a compound of formula (VII), which has an acetal group as a biodegradable group in at least one tail:



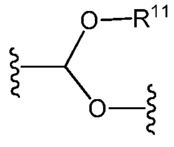
Formula (VII)

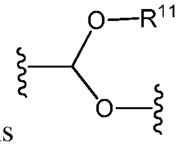
or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

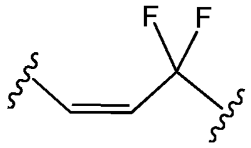
R', R^1, R^2, R, R^3, R^4, R^{10}, Q, R^5, a, and b are defined as in formula (I);

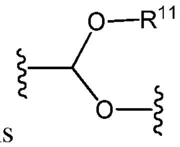
X and Y are each, independently, alkylene (e.g., C_6-C_8 alkylene) or alkenylene, wherein the alkylene or alkenylene group is optionally substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., );

M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -

$OSi(R^5)_2O$-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or  (wherein R^{11} is a C_4-C_{10} alkyl or C_4-C_{10} alkenyl));

with the proviso that at least one of M^1 and M^2 is  ; and

Z^1 and Z^2 are each, independently, C_4-C_{14} alkyl or C_4-C_{14} alkenyl, wherein the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2 (e.g., ).

In one embodiment, each of M^1 and M^2 is  .

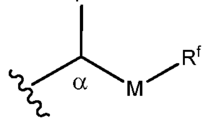
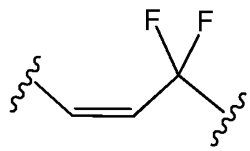
In another embodiment, X and Y are each, independently –$(CH_2)_n$– wherein n is 4 to 20, e.g., 4 to 18, 4 to 16, or 4 to 12. In one embodiment, n is 4, 5, 6, 7, 8, 9, or 10. In one exemplary embodiment, X and Y are -$(CH_2)_6$-. In another embodiment, X and Y are -$(CH_2)_7$-. In yet another embodiment, X and Y are -$(CH_2)_9$-. In yet another embodiment, X and Y are -$(CH_2)_8$-.

The $R'R^1R^2N$-$(R)_a$-Q-$(R)_b$- group can be any of the head groups described herein, including those shown in Table 1 below, and salts thereof. In one preferred embodiment, $R'R^1R^2N$-$(R)_a$-Q-$(R)_b$- is $(CH_3)_2N$-$(CH_2)_3$-C(O)O-, $(CH_3)_2N$-$(CH_2)_2$-NH-C(O)O-, $(CH_3)_2N$-$(CH_2)_2$-OC(O)-NH-, or $(CH_3)_2N$-$(CH_2)_3$-$C(CH_3)$=N-O-.

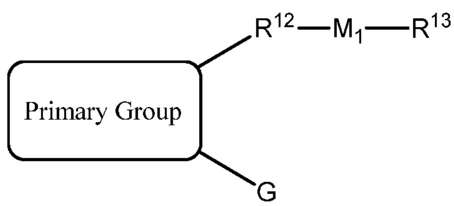
In another embodiment, the present invention relates to a cationic lipid or a salt thereof having:

(i) a central carbon atom,

(ii) a nitrogen containing head group directly bound to the central carbon atom, and

(iii) two hydrophobic tails directly bound to the central carbon atom, wherein each hydrophobic tail is of the formula $-R^e$-M-R^f where R^e is a C_4-C_{14} alkyl or alkenyl, M is a biodegradable group, and R^f is a branched alkyl or alkenyl (e.g., a C_{10}-C_{20} alkyl or C_{10}-C_{20} alkenyl), such that (i) the chain length of $-R^e$-M-R^f is at most 20 atoms (i.e. the total length of the tail from the first carbon atom after the central carbon atom to a terminus of the tail is at most 20), and (ii) the group $-R^e$-M-R^f has at least 20 carbon atoms (e.g., at least 21 atoms). Optionally, the alkyl or alkenyl group in R^e may be substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., ). Also, optionally, the alkenyl group in R^f may be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of R^f (e.g., ).

In one embodiment, the cationic lipid of the present invention (such as of formulas I-VII) has assymetrical hydrophobic groups (i.e., the two hydrophobic groups have different chemical formulas). For example, the cationic lipid can have the formula:

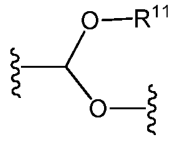


Formula (VIII)

or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), wherein

G is branched or unbranched C_3-C_{15} alkyl, alkenyl or alkynyl (e.g., a n-C_8 alkyl n-C_9 alkyl, or n-C_{10} alkyl);

R^{12} is a branched or unbranched alkylene or alkenylene (e.g., C_6-C_{20} alkylene or C_6-C_{20} alkenylene such as C_{12}-C_{20} alkylene or C_{12}-C_{20} alkenylene);

M_1 is a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or 

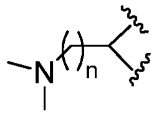
(wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

R^3 and R^4 are defined as in formula (I);

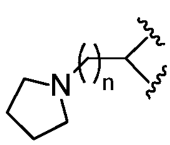
each occurrence of R^5 is, independently, H or alkyl (e.g., C_1-C_4 alkyl);

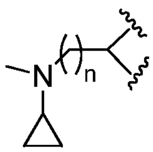
R^{13} is branched or unbranched C_3-C_{15} alkyl, alkenyl or alkynyl; [Primary Group] comprises a protonatable group having a pK_a of from about 4 to about 13, more preferably from about 5 to about 8 (e.g. from about 5 to about 7, or from about 5 to about 6.5, or from about 5.5 to about 6.5, or from about 6 to about 6.5).

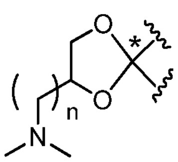
In one embodiment, the primary group includes (i) a head group, and (ii) a central moiety (e.g., a central carbon atom) to which both the hydrophobic tails are directly bonded. Representative central moieties include, but are not limited to, a central carbon atom, a central nitrogen atom, a central carbocyclic group, a central aryl group, a central hetrocyclic group (e.g., central tetrahydrofuranyl group or central pyrrolidinyl group) and a central heteroaryl group.

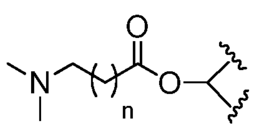
Representative [Primary Group]'s include, but are not limited to, 

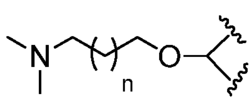
;



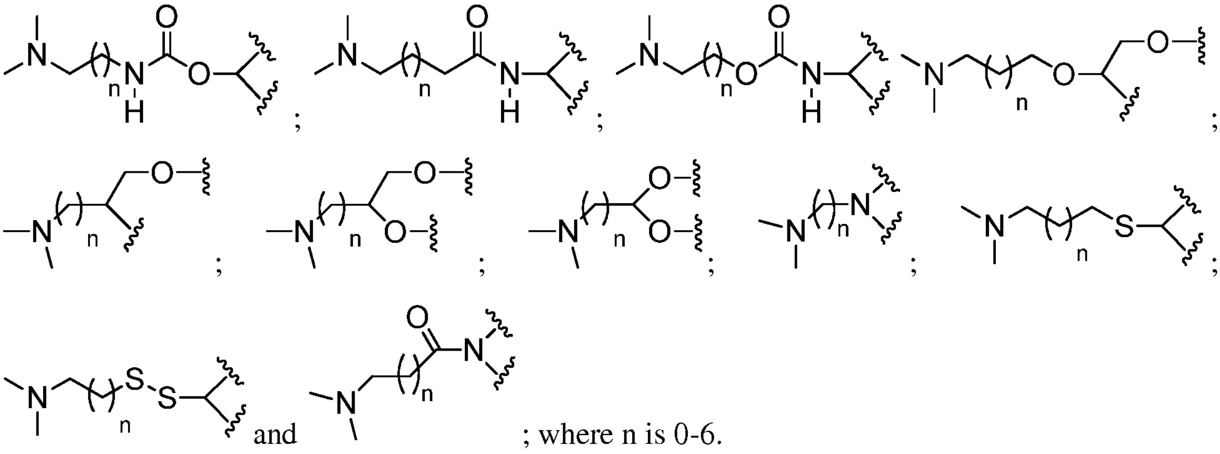
; 

; 

; 

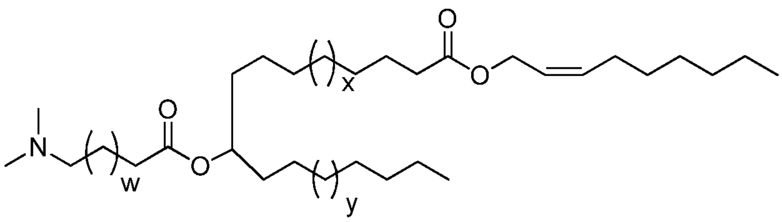
; 

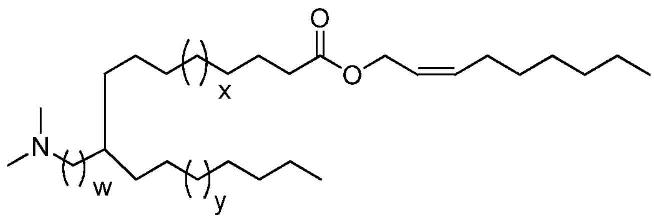
;

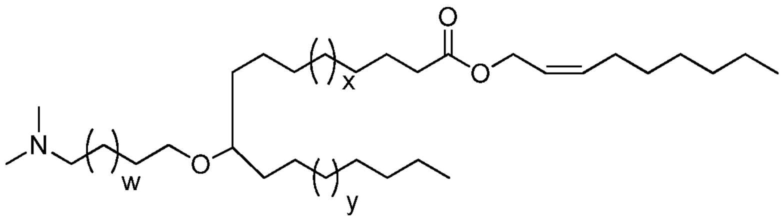


; where n is 0-6.

Representative asymmetrical cationic lipids include:







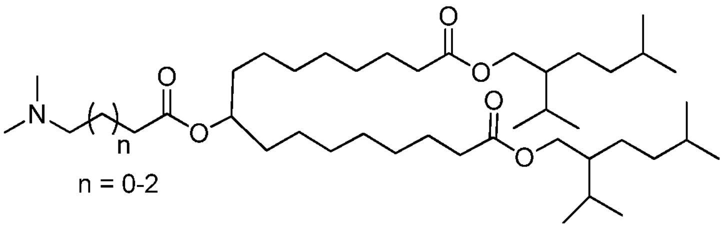
wherein w is 0, 1, 2, or 3; and x and y are each independently 1, 2, 3, 4, 5, 6, or 7.

In a preferred embodiment of the aforementioned biodegradable cationic lipids, the biodegradable cationic lipid has a logP value of at least 10.1 (as calculated by the software available at http://www.molinspiration.com/services/logp.html from Molinspiration

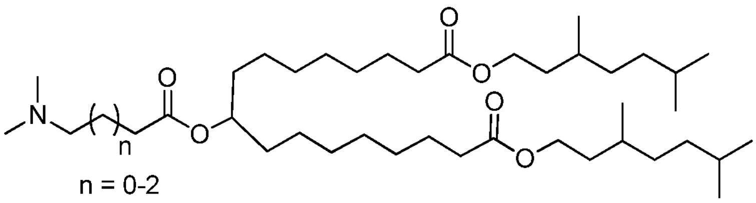
Cheminformatics of Slovensky Grob, Slovak Republic). More preferably, the logP value is at least 10.2 or 10.3.

In another preferred embodiment of the aforementioned biodegradable cationic lipids, the biodegradable cationic lipid in the lipid nanoparticle has a HPLC retention time (relative to the retention time of cholesterol in the lipid nanoparticle), hereafter referred to as $t_{lipid} - t_{chol}$, of at least 1.4. (The HPLC parameters are provided in the examples below. Unless otherwise specified, the formulation of the lipid nanoparticle used is that described in Example 31). More preferably, the $t_{lipid} - t_{chol}$ value is at least 1.75, 2.0, or 2.25.

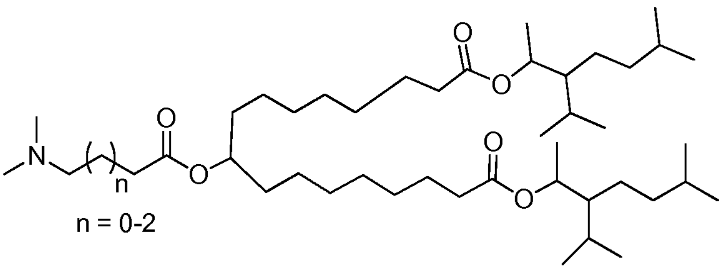
In another embodiment, the biodegradable cationic lipid of the present invention is not one selected from:



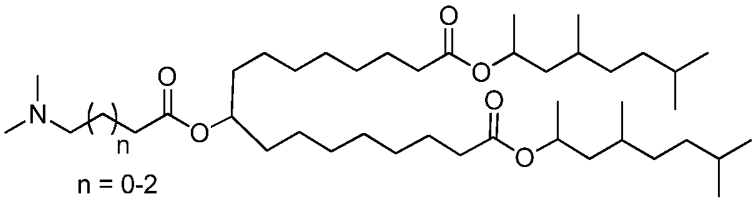
n = 0-2



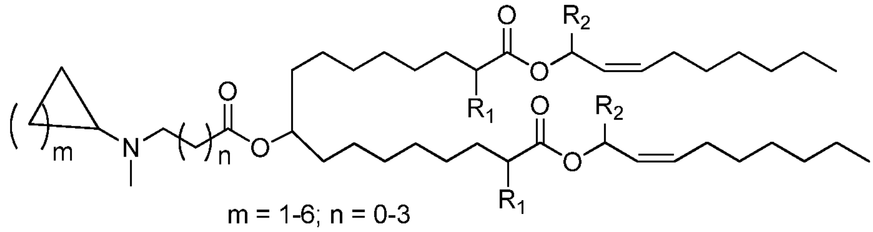
n = 0-2



n = 0-2

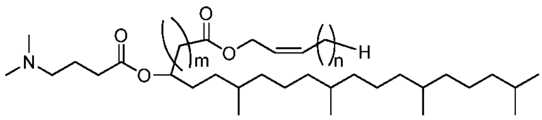


n = 0-2

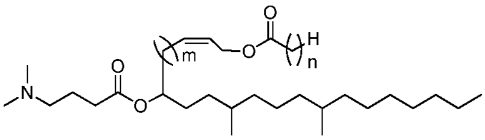


m = 1-6; n = 0-3

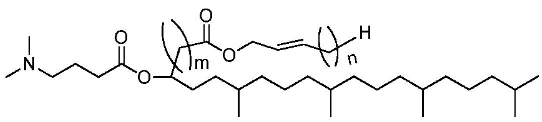
$R_1 = R_2$ = Me, Et, iPr etc.



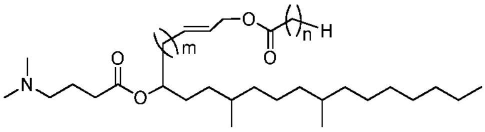
where m and n are integers, and m + n = 13



where m and n are integers, and m + n = 13



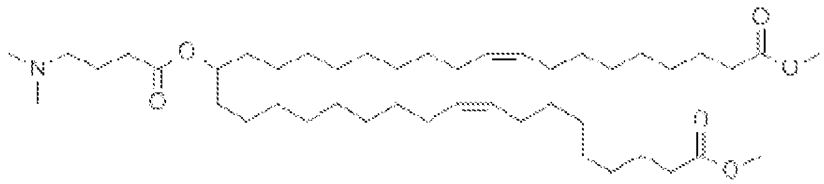
where m and n are integers, and m + n = 13

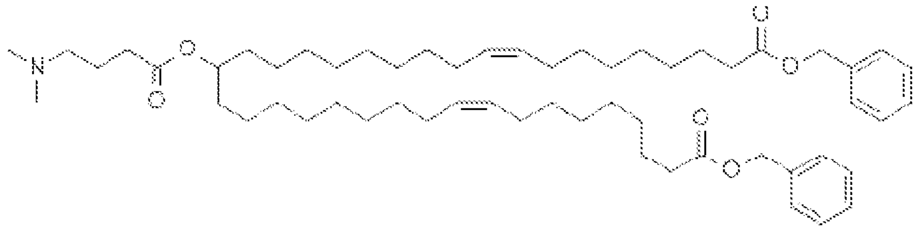


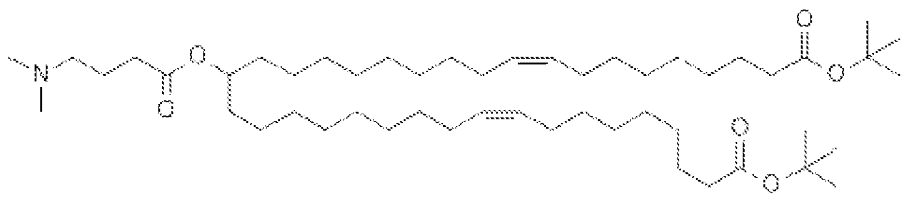
where m and n are integers, and m + n = 13

In yet another embodiment, the biodegradable cationic lipid is not one selected from those disclosed in International Publication No. WO 2011/153493 and U.S. Patent Publication No. 2012/0027803, both of which are hereby incorporated by reference.

Yet another embodiment is a biodegradable cationic lipid having (i) a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4, and (2) one or more biodegradable groups (such as an ester group) located in the mid- or distal section of a lipidic moiety (e.g., a hydrophobic chain) of the cationic lipid, with the proviso that the compound is not selected from







In another embodiment, the biodegradable cationic lipid is not one selected from those disclosed in International Publication No. WO 2011/153493 and U.S. Patent Publication No. 2012/0027803, both of which are hereby incorporated by reference. The incorporation of the biodegradable group(s) into the cationic lipid results in faster metabolism and removal of the cationic lipid from the body following delivery of the active pharmaceutical ingredient to a target area. In a preferred embodiment, the cationic lipid includes a branched alkyl or branched alkenyl group in its biodegradable group(s). In another preferred embodiment, the cationic lipd has a logP of at least 10.2 or 10.3. In yet another preferred embodiment, the cationic lipid has a $t_{lipid} - t_{chol}$, of at least 1.75, 2.0, or 2.25. The cationic lipid preferably has a pKa of from about 4 to about 7 (such as 6.0 to 6.5).

In one embodiment, the cationic lipid having a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4 comprises (a) a head group (preferably a nitrogen containing head group, such as the head groups described herein), (b) at least two hydrophobic tails, each of the formula –(hydrophobic chain)-(biodegradable group)-(hydrophobic chain), and (c) a linker group (for instance, a single central carbon atom) which is bound to the head group and the hydrophobic tails. The cationic lipid preferably has one, two, three, four or more of the properties listed below:

(i) a pKa of from about 4 to about 7 (such as 6.0 to 6.5);

(ii) in at least one hydrophobic tail (and preferably all hydrophobic tails), the biodegradable group is separated from the terminus of the hydrophobic tail by from about 6 to about 12 carbon atoms (for instance, 6 to 8 carbon atoms or 8 to 12 carbon atoms),

(iii) for at least one hydrophobic tail (and preferably all hydrophobic tails), the chain length from the linker group to the terminus of the hydrophobic tail is at most 21 (e.g., at most 20, or from about 17 to about 21, from about 18 to about 20, or from about 16 to about 18) (The atom(s) in the linker group are not counted when calculating the chain length.);

(iv) for at least one hydrophobic tail (and preferably all hydrophobic tails), the total number of carbon atoms in the hydrophobic tail is from about 17 to about 26 (such as from about 19 to about 26, or from about 21 to about 26);

(v) for at least one hydrophobic tail (and preferably all hydrophobic tails), the number of carbon atoms between the linker group and the biodegradable group ranges from about 5 to about 10 (for example, 6 to 10, or 7 to 9);

(vi) for at least one hydrophobic tail (and preferably all hydrophobic tails), the total number of carbon atoms between the linker group and the terminus of the hydrophobic tail is from about 15 to about 20 (such as from 16 to 20, 16 to 18, or 18 to 20);

(vii) for at least one hydrophobic tail (and preferably all hydrophobic tails), the total number of carbon atoms between the biodegradable group and the terminus of the hydrophobic tail is from about 12 to about 18 (such as from 13 to 25);

(viii) for at least one hydrophobic tail (and preferably all hydrophobic tails), the terminal hydrophobic chain in the hydrophobic tail is a branched alkyl or alkenyl group, for example, where the branching occurs at the α, β, γ, or δ position on the hydrophobic chain relative to the biodegradable group;

(ix) when formulated as a lipid nanoparticle (such as in Example 35), the cationic lipid has an *in vivo* half life ($t_{1/2}$) in the liver of less than about 3 hours, such as less than about 2.5 hours, less than about 2 hours, less than about 1.5 hours, less than about 1 hour, less than about 0.5 hour or less than about 0.25 hours;

(x) when formulated as a lipid nanoparticle (such as in Example 35), the cationic lipid is eliminated from the liver in mice with a greater than 10-fold reduction in lipid levels relative to C_{max} within the first 24 hours post-dose;

(xi) when formulated as a lipid nanoparticle (such as in Example 35), the cationic lipid is eliminated from the spleen in mice with an equal or greater than 10-fold reduction in lipid levels relative to C_{max} within the first 168 hours post-dose; and

(xii) when formulated as a lipid nanoparticle (such as in Example 35), the cationic lipid is eliminated from plasma with a terminal plasma half-life (t1/2β) in rodents and non-human primates of 48 hours or shorter.

The present invention embodies compounds having any combination of some or all of the aforementioned properties. These properties provide a cationic lipid which remains intact until delivery of an active agent, such as a nucleic acid, after which cleavage of the hydrophobic tail occurs *in vivo*. For instance, the compounds can have all of properties (i) to (viii) (in addition to the logP or $t_{lipid} - t_{chol}$ value). In another embodiment, the compounds have properties (i), (ii), (iii), and (viii). In yet another embodiment, the compounds have properties (i), (ii), (iii), (v), (vi), and (viii).

Another embodiment is a method of preparing a cationic lipid comprising:

(a) designing a cationic lipid having a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4, and optionally also having one, two, three, four, or more properties from the list above (i.e., properties (i)-(xii)); and

(b) synthesizing the cationic lipid of step (a). The cationic lipid in step (a) may comprises (a) a head group (preferably a nitrogen containing head group, such as the head groups described herein), (b) at least two hydrophobic tails, each of the formula –(hydrophobic chain)-(biodegradable group)-(hydrophobic chain), and (c) a linker group (for instance, a single central carbon atom) which is bound to the head group and the hydrophobic tails. Step (a) may comprise:

(a)(i) preparing one or more cationic lipids having a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4, and optionally also having one, two, three, four, or more properties from the list above (i.e., properties (i)-(xii);

(a)(ii) screening the cationic lipids to determine their efficacy and/or toxicity in lipid nanoparticles; and

(a)(iii) selecting a cationic lipid for synthesis.

Yet another embodiment is a method of designing a cationic lipid comprising:

(a) selecting a cationic lipid having a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4, and optionally also having one, two, three, four, or more properties from the list above (i.e., properties (i)-(xii)); and

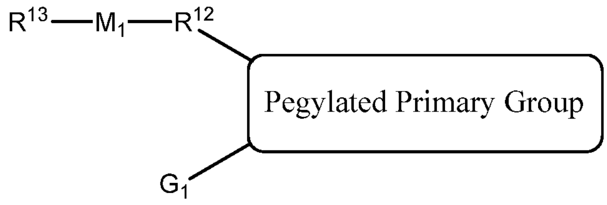
(b) optionally,

(i) preparing one or more cationic lipids having a logP value of at least 10.1 and/or a $t_{lipid} - t_{chol}$, of at least 1.4, and optionally also having one, two, three, four, or more properties from the list above (i.e., properties (i)-(xii);

(ii) screening the cationic lipids to determine their efficacy and/or toxicity in lipid nanoparticles; and

(iii) optionally, selecting a cationic lipid for further development or use.

In one embodiment, the PEG lipid has the formula:

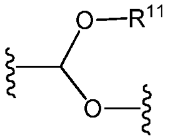


Formula (IX)

wherein

G_1 is branched or unbranched C_3-C_{15} alkyl, alkenyl or alkynyl (e.g., a n-C_8 alkyl n-C_9 alkyl, or n-C_{10} alkyl); or G_1 is $-R^{12}$-M_1-R^{13};

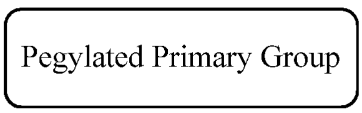
R^{12} is a branched or unbranched alkylene or alkenylene (e.g., C_6-C_{20} alkylene or C_6-C_{20} alkenylene such as C_{12}-C_{20} alkylene or C_{12}-C_{20} alkenylene);

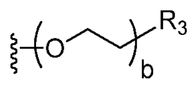
M_1 is a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or  (wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

R^3 and R^4 are defined as in formula (I);

each occurrence of R^5 is, independently, H or alkyl (e.g., C_1-C_4 alkyl);

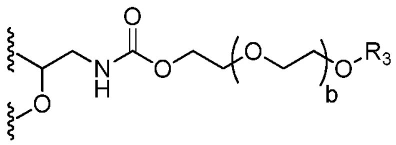
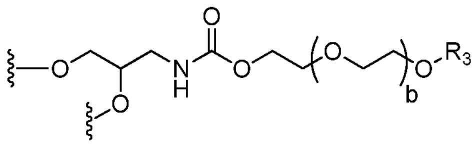
R^{13} is branched or unbranched C_3-C_{15} alkyl, alkenyl or alkynyl;

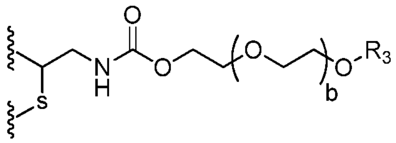
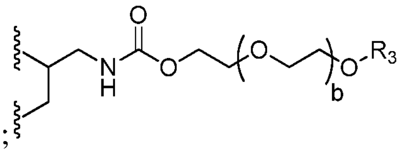
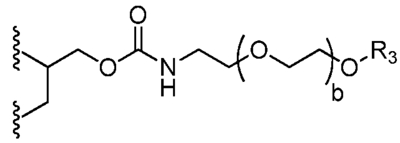


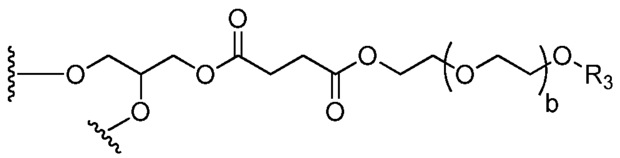
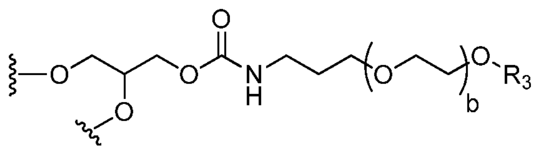
comprises a PEG moiety, such as  moiety wherein b is an integer from 10 to 1,000 (e.g., 5-100, 10-60, 15-50, or 20-45); R^3 is -H, -R^c, or -OR^c; and R^c is -H, alkyl, acyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl.

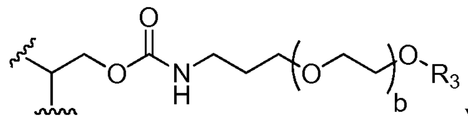
In one embodiment, the pegylated primary group includes (i) a head group having a PEG moiety, and (ii) a central moiety (e.g., a central carbon atom) to which both the hydrophobic tails are directly bonded. Representative central moieties include, but are not limited to, a central carbon atom, a central nitrogen atom, a central carbocyclic group, a central aryl group, a central hetrocyclic group (e.g., central tetrahydrofuranyl group or central pyrrolidinyl group) and a central heteroaryl group.

Representative [Pegylated Primary Group]'s include, but are not limited to,

 ;  ;

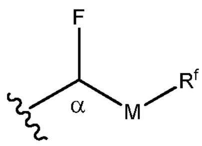
 ;  ;  ;

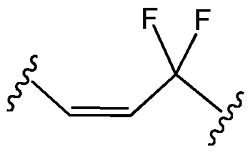
 ;  ;

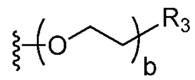
 where b is 10-100 (e.g., 20-50 or 40-50)

Another embodiment of the present invention is a PEG lipid (or a salt thereof) having:

(i) a pegylated primary group including a head group which includes a PEG moiety (e.g., having from 10 to 1000 repeating units such as ethoxy units)), and

(iii) one or more hydrophobic tails (preferably, two hydrophobic tails) directly bound to the pegylated primary group, wherein at least one hydrophobic tail is of the formula $-R^e-M-R^f$ where R^e is a C_4-C_{14} alkyl or alkenyl, M is a biodegradable group, and R^f is a branched alkyl or alkenyl (e.g., a C_{10}-C_{20} alkyl or C_{10}-C_{20} alkenyl), such that (i) the chain length of $-R^e-M-R^f$ is at most 20 atoms (i.e. the total length of the tail from the first carbon atom after the central carbon atom to a terminus of the tail is at most 20), and (ii) the group $-R^e-M-R^f$ has at least 20 carbon atoms (e.g., at least 21 atoms). Optionally, the alkyl or alkenyl group in R^e may be substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group (e.g., 

). Also, optionally, the alkenyl group in R^f may be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of R^f (e.g., 

). In one embodiment, the pegylated primary group includes (i) a head group having a PEG moiety, and (ii) a central moiety (e.g., a central carbon atom) to which the hydrophobic tails are directly bound. The PEG moiety may have 5-100, 10-60, 15-50, or 20-45 repeating units. For example, the PEG moiety may have the formula 

moiety wherein b is an integer from 10 to 1,000 (e.g., 5-100, 10-60, 15-50, or 20-45); R^3 is -H, -R^c, or -OR^c; and R^c is -H, alkyl (e.g., C_1-C_4 alkyl), acyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, or heterocyclyl.

Yet another embodiment is a lipid particle that includes a cationic lipid and/or PEG lipid of the present invention. In one embodiment, the lipid particle includes a cationic lipid of the present invention (e.g., of one of formulas (I)-(VIII)). In another embodiment, the lipid particle includes a PEG lipid of the present invention (e.g., of formula (IX)). In yet another embodiment, the lipid particle includes a cationic lipid of the present invention and a PEG lipid of the present invention.

In a preferred embodiment, the lipid particle includes a neutral lipid, a lipid capable of reducing aggregation, a cationic lipid, and optionally, a sterol (e.g., cholesterol). Suitable neutral lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), POPC, DOPE, and SM. Suitable lipids capable of reducing aggregation include, but are not limited to, a PEG lipid, such as PEG-DMA, PEG-DMG, and those of the present invention (e.g., of formula (IX)) or a combination thereof.

The lipid particle may further include an active agent (e.g., a therapeutic agent). The active agent can be a nucleic acid such as a plasmid, an immunostimulatory oligonucleotide, an siRNA, an antisense oligonucleotide, a microRNA, an antagomir, an aptamer, or a ribozyme. In a preferred embodiment, the nucleic acid is a siRNA. In another preferred embodiment, the nucleic acid is a miRNA.

In another embodiment, the lipid particle includes a cationic lipid of the present invention, a neutral lipid and a sterol. The lipid particle may further include an active agent, such as a nucleic acid (e.g., an siRNA or miRNA).

In yet another embodiment, the lipid particle includes a PEG lipid of the present invention, a cationic lipid, a neutral lipid, and a sterol. The lipid particle may further include an active agent, such as a nucleic acid (e.g., an siRNA or miRNA).

The lipid particles described herein may be lipid nanoparticles.

Yet another embodiment of the invention is a pharmaceutical composition which includes a lipid particle of the present invention and a pharmaceutically acceptable carrier.

In one embodiment, the cationic lipid remains intact until delivery of the nucleic acid molecule after which cleavage of the hydrophobic tail occurs *in vivo*.

In another embodiment, the PEG lipid remains intact until delivery of the nucleic acid molecule after which cleavage of the hydrophobic tail occurs *in vivo*.

In another embodiment, the present invention relates to a method of delivering a nucleic acid molecule comprising administering a nucleic lipid particle comprising (i) the nucleic acid molecule and (ii) a cationic lipid and/or a PEG lipid of the present invention. In one embodiment, the cationic lipid and/or a PEG lipid remains intact until delivery of the nucleic acid molecule after which cleavage of the hydrophobic tail occurs *in vivo*.

Yet another aspect is a method of modulating the expression of a target gene in a cell by providing to the cell a lipid particle of the present invention. The active agent can be a nucleic acid selected from a plasmid, an immunostimulatory oligonucleotide, an siRNA, an antisense oligonucleotide, a microRNA, an antagomir, an aptamer, and a ribozyme. In a preferred embodiment, the nucleic acid is a siRNA or miRNA.

Yet another aspect is a method of treating a disease or disorder characterized by the overexpression of a polypeptide in a subject by providing to the subject a pharmaceutical composition of the present invention, wherein the active agent is a nucleic acid selected from an siRNA, a microRNA, and an antisense oligonucleotide, and wherein the siRNA, microRNA, or antisense oligonucleotide includes a polynucleotide that specifically binds to a polynucleotide that encodes the polypeptide, or a complement thereof. In a preferred embodiment, the nucleic acid is a siRNA or miRNA.

Yet another aspect is a method of treating a disease or disorder characterized by underexpression of a polypeptide in a subject by providing to the subject a pharmaceutical composition of the present invention, wherein the active agent is a plasmid that encodes the polypeptide or a functional variant or fragment thereof.

Yet another aspect is a method of inducing an immune response in a subject by providing to the subject a pharmaceutical composition wherein the active agent is an immunostimulatory oligonucleotide.

Yet another aspect is a transfection agent that includes the composition or lipid particles described above, where the composition or lipid particles include a nucleic acid. The agent, when contacted with cells, can efficiently deliver nucleic acids to the cells. Yet another aspect is a method of delivering a nucleic acid to the interior of a cell, by obtaining or forming a composition or lipid particles described above, and contacting the composition or lipid particles with a cell.

Detailed Description

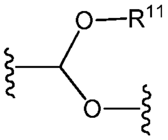
In one aspect, the present invention relates to a lipid particle that includes a neutral lipid, a lipid capable of reducing aggregation (e.g., a PEG lipid), a cationic lipid, and optionally a sterol. In certain embodiments, the lipid particle further includes an active agent (e.g., a therapeutic agent). Various exemplary embodiments of these lipids, lipid particles and compositions comprising the same, and their use to deliver therapeutic agents and modulate gene and protein expression are described in further detail below.

The Cationic Lipid

In one embodiment, the cationic lipid is a compound of any one of Formulas I-VIII. The following disclosure represents various embodiments of the compounds described above, including the compounds of Formulas I-VIII.

In one embodiment, M^1 and M^2 are each, independently:

-OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or



(wherein R^{11} is a C_2-C_8 alkyl or alkenyl).

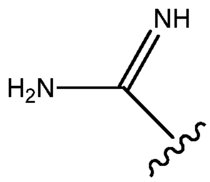
In another embodiment, M^1 and M^2 are each, independently:

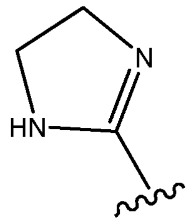
-OC(O)-, -C(O)-O-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -O-C(O)O-, -C(O)N(R^5)-, -N(R^5)C(O)-, -C(O)S-, -SC(O)-, -C(S)O-,-OC(S)-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, or -OC(O)(CR^3R^4)C(O)-.

In yet another embodiment, M^1 and M^2 are each, independently:

-C(O)-O-, -OC(O)-, -C(R^5)=N-, -C(R^5)=N-O-, -O-C(O)O-, -C(O)N(R^5)-, -C(O)S-, -C(S)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, or -OC(O)(CR^3R^4)C(O)-.

In another embodiment, M^1 and M^2 are each –C(O)O-.

In one embodiment, R^1 and R^2 are each, individually, optionally substituted alkyl, cycloalkyl, cycloalkylalkyl, or heterocycle. In one embodiment, R^1 is alkyl and R^2 is alkyl, cycloalkyl or cycloalkylalkyl. In one embodiment, R^1 and R^2 are each, individually, alkyl (e.g., C_1-C_4 alkyl, such as methyl, ethyl, or isopropyl). In one embodiment, R^1 and R^2 are both methyl. In another embodiment, R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring (e.g., N-methylpiperazinyl). In another embodiment, one of R^1 and R^2 is 

or 

(e.g., R^1 is one of the two aforementioned groups and R^2 is hydrogen).

In one embodiment, R' is hydrogen or alkyl. In another embodiment, R' is hydrogen or methyl. In one embodiment, R' is absent. In one embodiment, R' is absent or methyl.

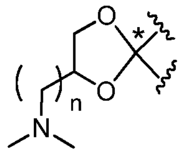
For cationic lipid compounds which contain an atom (e.g., a nitrogen atom) that carries a positive charge, the compound also contains a negatively charged counter ion. The counterion can be any anion, such as an organic or inorganic anion. Suitable examples of anions include, but are not limited to, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate, α-glycerophosphate, halide (e.g., chloride), sulfate, nitrate, bicarbonate, and carbonate. In one embodiment, the counterion is a halide (e.g., Cl).

In one embodiment each R is, independently, $-(CR^3R^4)-$, wherein R^3 and R^4 are each, independently, H or alkyl (e.g., C_1-C_4 alkyl). For example, in one embodiment each R is, independently, $-(CHR^4)-$, wherein each R^4 is, independently H or alkyl (e.g., C_1-C_4 alkyl). In another embodiment, each R is, independently, $-CH_2-$, $-C(CH_3)_2-$ or $-CH(iPr)-$ (where *i*Pr is isopropyl). In another embodiment, each R is $-CH_2-$.

In another embodiment R^5 is, in each case, hydrogen or methyl. For example, R^5 can be, in each case, hydrogen.

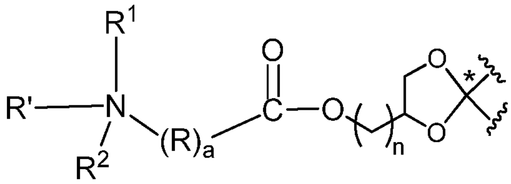
In one embodiment, Q is absent, -C(O)O-, -OC(O)-, -C(O)N(R^5)-, -N(R^5)C(O)-, -S-S-, -OC(O)O-, -C(R^5)=N-O-, -OC(O)N(R^5)-, -N(R^5)C(O)N(R^5)-, -N(R^5)C(O)O-, -C(O)S-, -C(S)O- or -C(R^5)=N-O-C(O)-. In one embodiment, Q is –C(O)O-.

In one embodiment, the dashed line to Q is absent, b is 0 and R'R^1R^2N-$(R)_a$-Q- and the tertiary carbon adjacent to it (C*) form the following group:



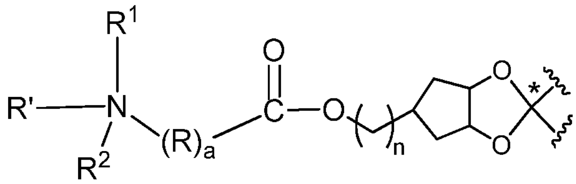
where n is 1 to 4 (e.g., n is 2).

In one embodiment, the dashed line to Q is absent, b is 0 and R'R^1R^2N-$(R)_a$-Q- and the tertiary carbon adjacent to it form the following group:

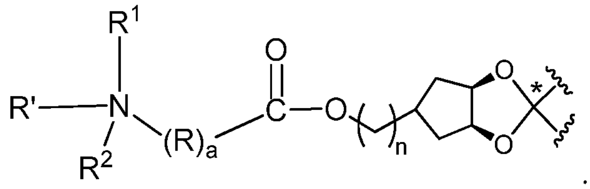


where n is 1 to 4 (e.g., n is 2), and R^1, R^2, R, a, and b are as defined with respect to formula (I). In one embodiment, a is 3.

In one embodiment, the dashed line to Q is absent, b is 0 and R'R^1R^2N-$(R)_a$-Q- and the tertiary carbon adjacent to it form the following group:



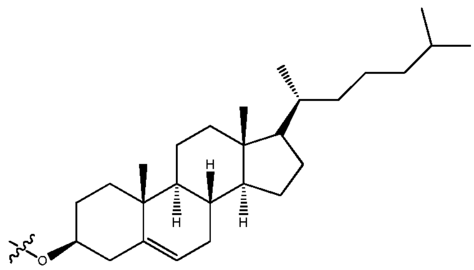
where n is 1 to 4 (e.g., n is 2), and R^1, R^2, R, a, and b are as defined with respect to formula (I). In one embodiment, a is 0. For example, the group can be:



In one embodiment, b is 0. In another embodiment, a is 2, 3, or 4 and b is 0. For example, in one embodiment, a is 3 and b is 0. In another embodiment, a is 3, b is 0, and Q is –C(O)O-.

In certain embodiments, the biodegradable group present in the cationic lipid is selected from an ester (e.g., -C(O)O- or –OC(O)-), disulfide (-S-S-), oxime (e.g., -C(H)=N-O- or –O-N=C(H)-), -C(O)-O-, -OC(O)-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -O-C(O)O-, -C(O)N(R^5), -N(R^5)C(O)-, -C(S)(NR^5)-, (NR^5)C(S)-, -N(R^5)C(O)N(R^5)-, -C(O)S-, -SC(O)-, -C(S)O-,-OC(S)-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, or -OC(O)(CR^3R^4)C(O)-.

A suitable cholesterol moiety for the cationic lipids of the present invention (including compounds of formulas I-VI) has the formula:

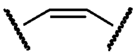
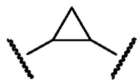


Additional embodiments include a cationic lipid having a head group, one or more hydrophobic tails, and a central moiety between the head group and the one or more tails. The head group can include an amine; for example an amine having a desired pK_a. The pK_a can be influenced by the structure of the lipid, particularly the nature of head group; e.g., the presence, absence, and location of functional groups such as anionic functional groups, hydrogen bond donor functional groups, hydrogen bond acceptor groups, hydrophobic groups (e.g., aliphatic groups), hydrophilic groups (e.g., hydroxyl or methoxy), or aryl groups. The head group amine can be a cationic amine; a primary, secondary, or tertiary amine; the head group can include one amine group (monoamine), two amine groups (diamine), three amine groups (triamine), or a larger number of amine groups, as in an oligoamine or polyamine. The head group can include a functional group that is less strongly basic than an amine, such as, for example, an imidazole, a pyridine, or a guanidinium group. The head group can be zwitterionic. Other head groups are suitable as well.

Representative central moieties include, but are not limited to, a central carbon atom, a central nitrogen atom, a central carbocyclic group, a central aryl group, a central hetrocyclic group (e.g., central tetrahydrofuranyl group or central pyrrolidinyl group) and a central heteroaryl group. Additionally, the central moiety can include, for example, a glyceride linker, an acyclic glyceride analog linker, or a cyclic linker (including a spiro linker, a bicyclic linker, and a polycyclic linker). The central moiety can include functional groups such as an ether, an ester, a phosphate, a phosphonate, a phosphorothioate, a sulfonate, a disulfide, an acetal, a ketal, an imine, a hydrazone, or an oxime. Other central moieties and functional groups are suitable as well.

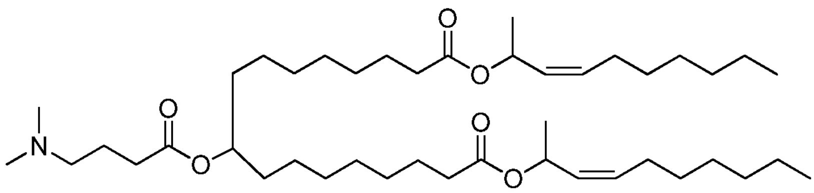
In one embodiment, the cationic lipid is a racemic mixture. In another embodiment, the cationic lipid is enriched in one diastereomer, e.g. the cationic lipid has at least 95%, at least 90%, at least 80% or at least 70% diastereomeric excess. In yet another embodiment, the cationic lipid is enriched in one enantiomer, e.g. the lipid has at least 95%, at least 90%, at least 80% or at least 70% enantiomer excess. In yet another embodiment, the cationic lipid is chirally pure, e.g. is a single optical isomer. In yet another embodiment, the cationic lipid is enriched for one optical isomer.

Where a double bond is present (e.g., a carbon-carbon double bond or carbon-nitrogen double bond), there can be isomerism in the configuration about the double bond (i.e. cis/trans or E/Z isomerism). Where the configuration of a double bond is illustrated in a chemical structure, it is understood that the corresponding isomer can also be present. The amount of isomer present can vary, depending on the relative stabilities of the isomers and the energy required to convert between the isomers. Accordingly, some double bonds are, for practical purposes, present in only a single configuration, whereas others (e.g., where the relative stabilities are similar and the energy of conversion low) may be present as inseparable equilibrium mixture of configurations.

In some cases, a double-bonded unsaturation is replaced by a cyclic unsaturation. The cyclic unsaturation can be a cycloaliphatic unsaturation, e.g., a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl group. In some cases, the cyclic group can be a polycyclic group, e.g., a bicyclic group or tricyclic group. A bicyclic group can be bridged, fused, or have a spiro structure. In some cases, a double bond moiety can be replaced by a cyclopropyl moiety, e.g.,  can be replaced by .

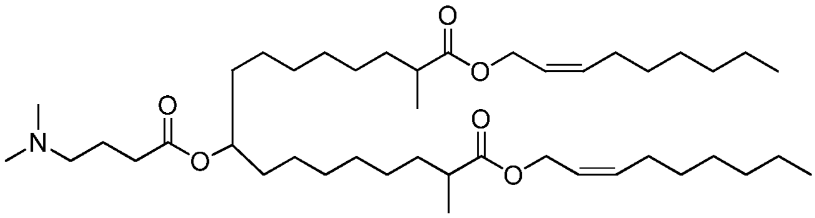
The cationic lipid includes one or more biodegradable groups. The biodegradable group(s) include one or more bonds that may undergo bond breaking reactions in a biological environment, e.g., in an organism, organ, tissue, cell, or organelle. Functional groups that contain a biodegradable bond include, for example, esters, dithiols, and oximes. Biodegradation can be a factor that influences the clearance of the compound from the body when administered to a subject. Biodegredation can be measured in a cell based assay, where a formulation including a cationic lipid is exposed to cells, and samples are taken at various time points. The lipid fractions can be extracted from the cells and separated and analyzed by LC-MS. From the LC-MS data, rates of biodegradation (e.g., as $t_{1/2}$ values) can be measured.

For example, the compound



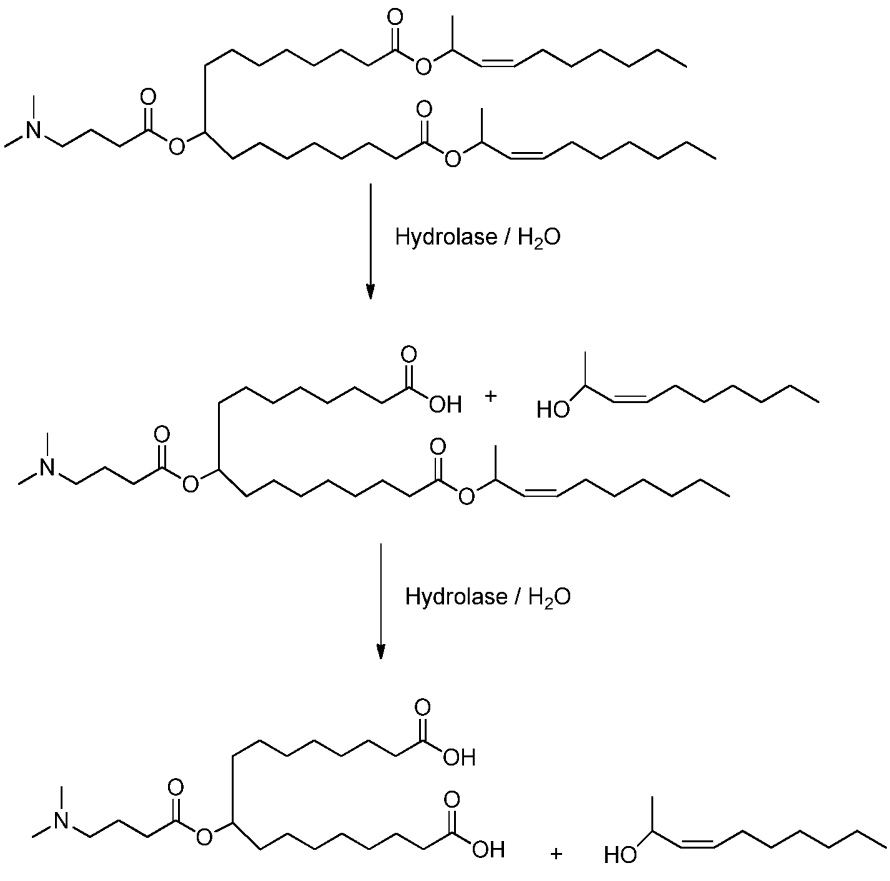
(Compound 1)

includes an ester linkage in each aliphatic chain, which can undergo hydrolysis in a biological environment, for example, when exposed to, e.g., a lipase or an esterase. The structure of the compound, of course, influences the rate at which the compound undergoes biodegradation. Thus, a compound where the methyl substituent is on the other side of the biodegradable group such as



would be expected to exhibit a different rate of biodegradation. Greater effects on that rate would be expected from changes in the structure of the compound at the site of hydrolysis. One modification that can influence the rate of hydrolysis, and thereby influence the rate of biodegradation and clearance from a subject's body, is to make the leaving group of the hydrolysis reaction have a secondary, rather than primary, alcohol.

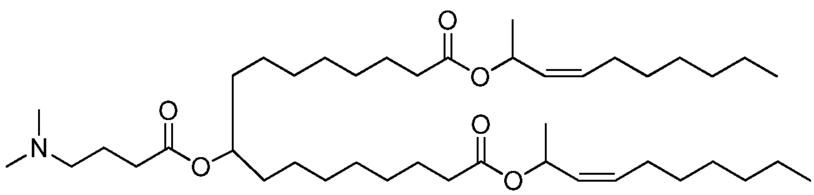
For example, without wishing to be bound by theory, Compound 1 shown above may be metabolized as shown in the scheme below:



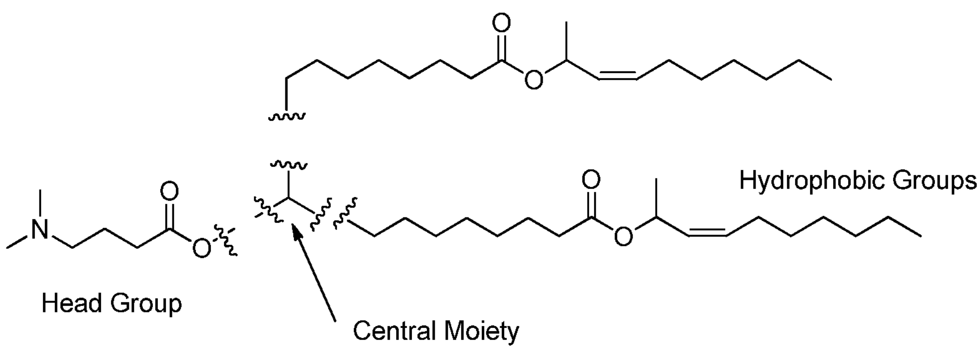
In one embodiment, a cationic lipid of any of the embodiments described herein has an *in vivo* half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 3 hours, such as less than about 2.5 hours, less than about 2 hours, less than about 1.5 hours, less than about 1 hour, less than about 0.5 hour or less than about 0.25 hours. The cationic lipid preferably remains intact, or has a half-life sufficient to form a stable lipid nanoparticle which effectively delivers the desired active pharmaceutical ingredient (e.g., a nucleic acid) to its target but thereafter rapidly degrades to minimize any side effects to the subject. For instance, in mice, the cationic lipid preferably has a $t_{1/2}$ in the spleen of from about 1 to about 7 hours.

In another embodiment, a cationic lipid of any of the embodiments described herein containing a biodegradable group or groups has an *in vivo* half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 10% (e.g., less than about 7.5%, less than about 5%, less than about 2.5%) of that for the same cationic lipid without the biodegrable group or groups.

Some cationic lipids can be conveniently represented as a hydrophobic group combined via a central moiety (such as a carbon atom) with a headgroup. By way of example, the compound:



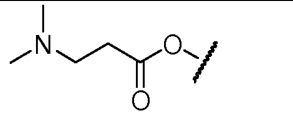
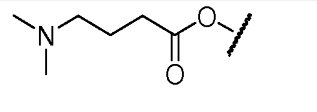
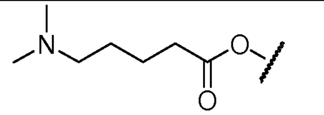
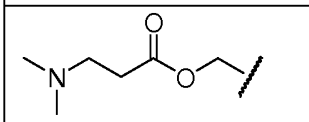
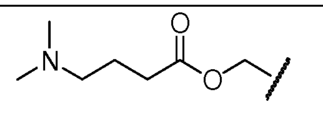
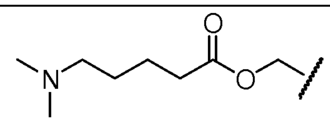
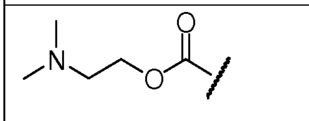
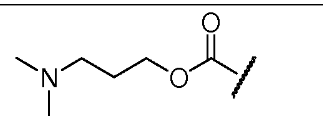
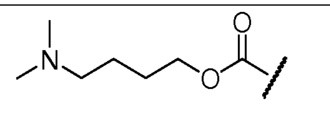
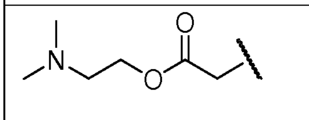
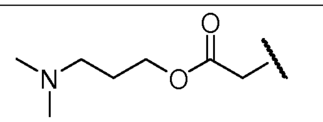
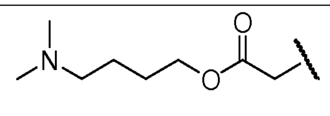
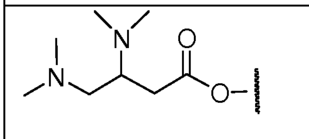
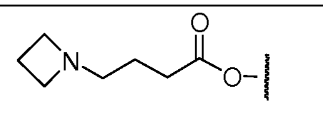
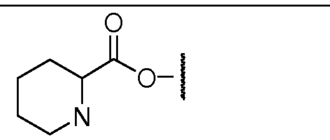
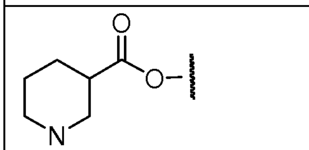
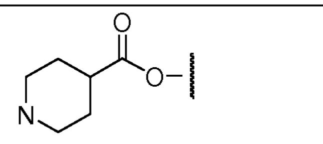
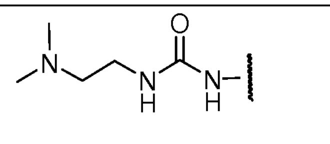
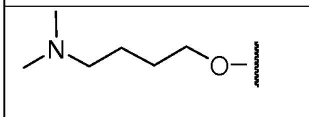
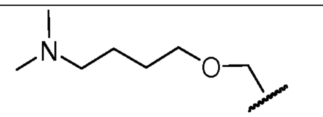
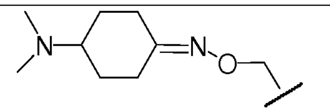
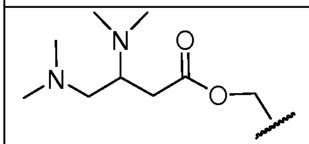
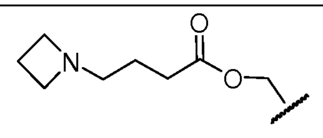
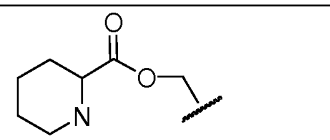
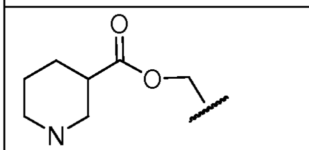
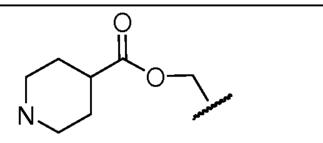
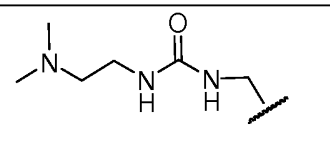
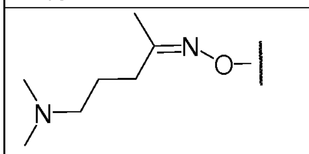
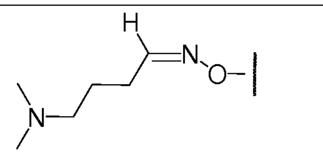
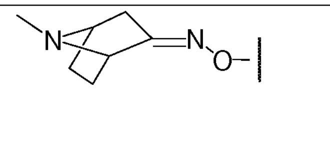
can be thought of as a combination of a headgroup, a central moiety, and two hydrophobic groups as follows:

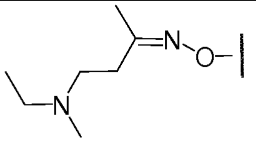
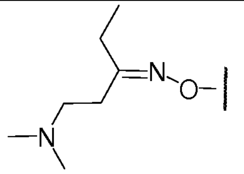
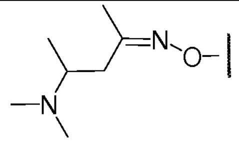
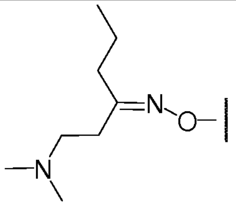
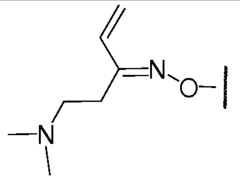
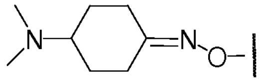
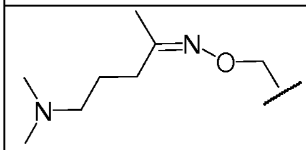
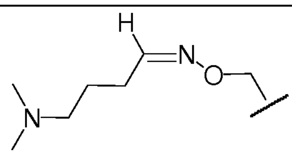
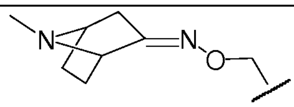
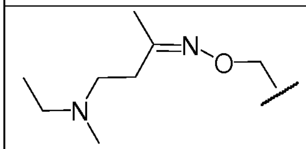
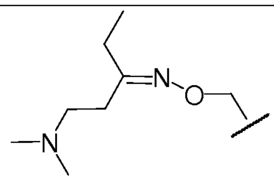
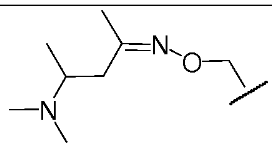
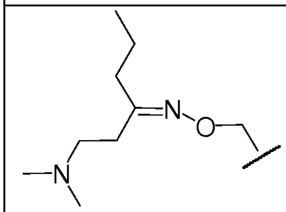
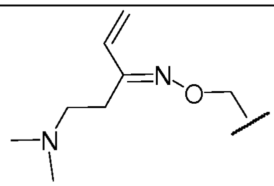
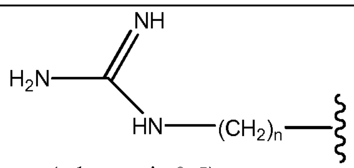
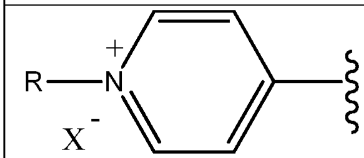
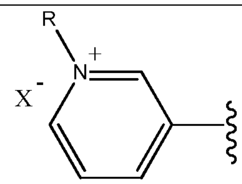
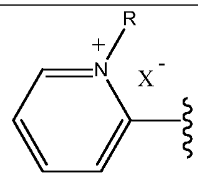
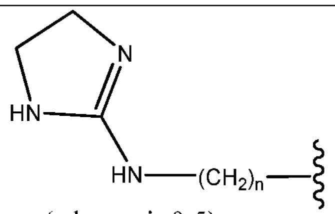


The present invention includes compounds composed of any combination of the head and hydrophobic groups listed below (in combination with a central moiety (such as a central carbon atom).

Some suitable head groups include those depicted in Table 1A:

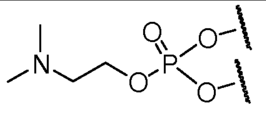
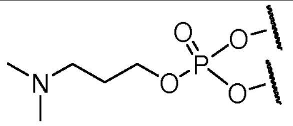
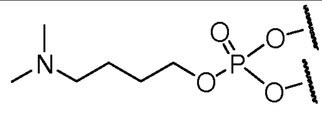
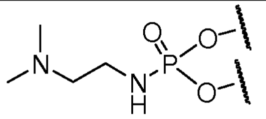
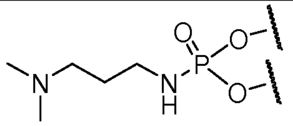
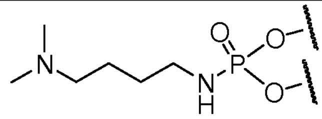
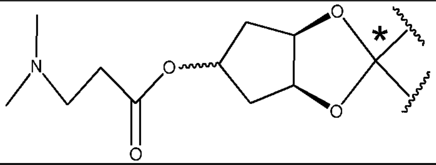
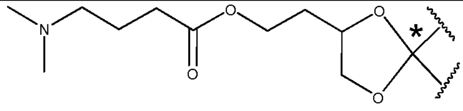
Table 1A

		
		
		
		
		 (where n is 0-5)
 R = H, alkyl (e.g., methyl) X = halogen (e.g., Cl)	 R = H, alkyl (e.g., methyl) X = halogen (e.g., Cl)	 R = H, alkyl (e.g., methyl) X = halogen (e.g., Cl)
 (where n is 0-5)		

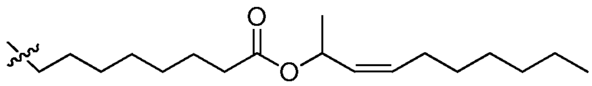
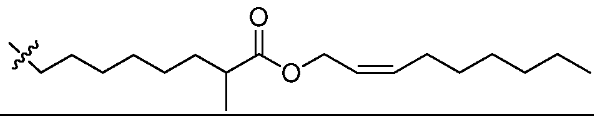
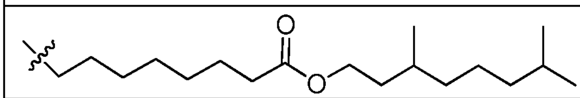
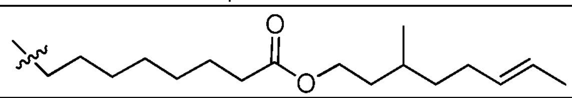
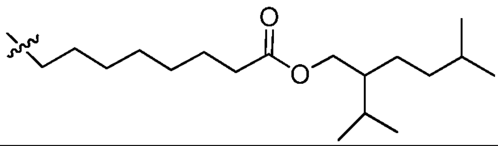
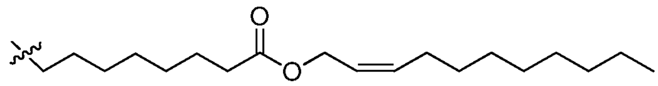
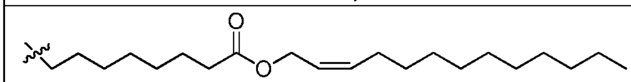
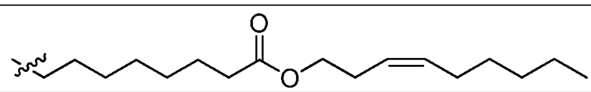
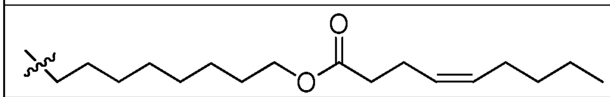
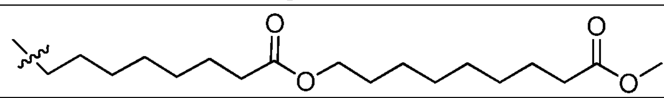
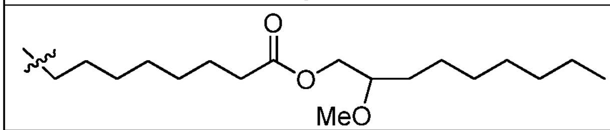
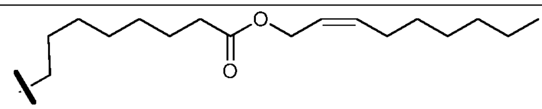
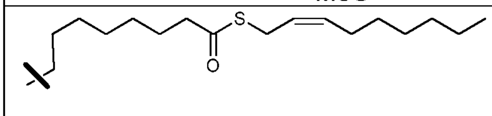
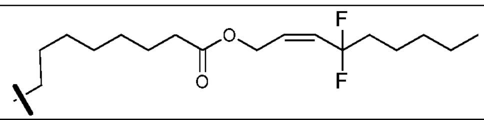
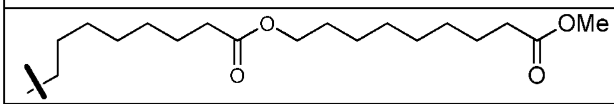
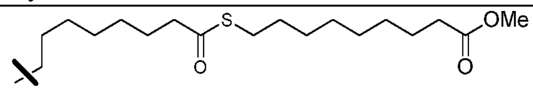
Suitable primary groups include, but are not limited to, those that are a combination of a head group from table 1A with a central carbon atom. Other suitable primary groups include those in table 1B below:

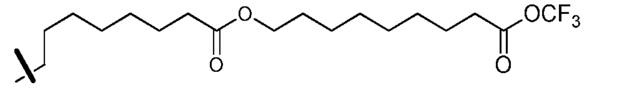
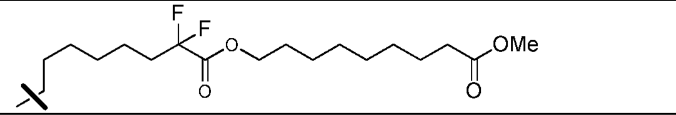
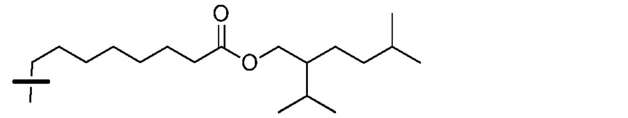
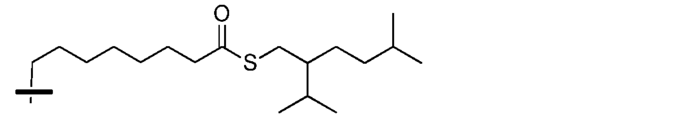
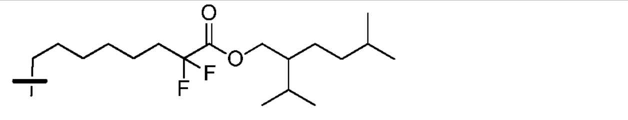
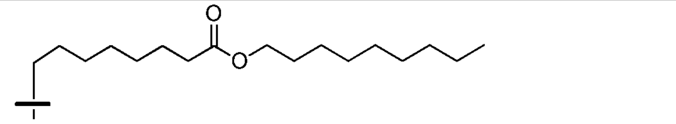
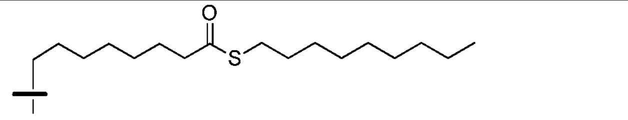
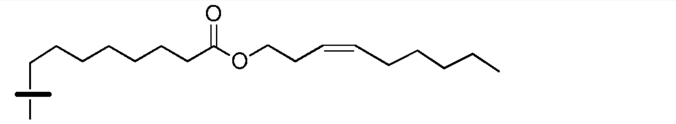
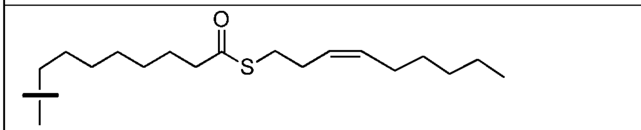
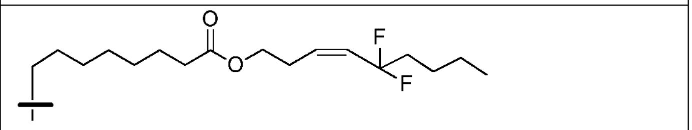
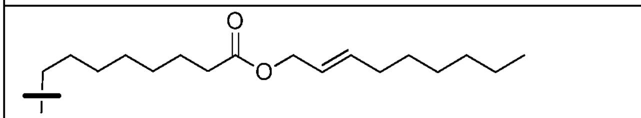
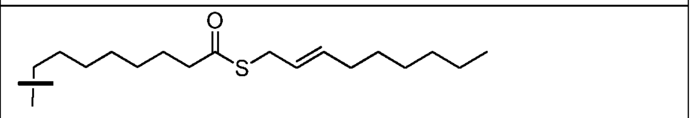
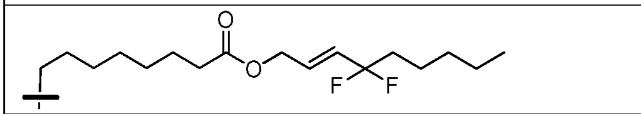
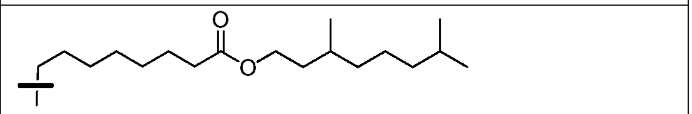
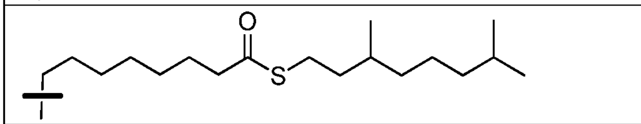
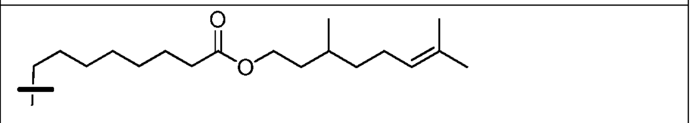
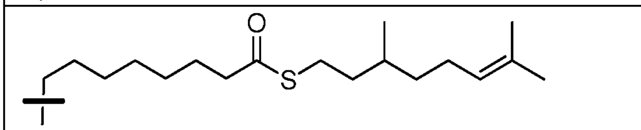
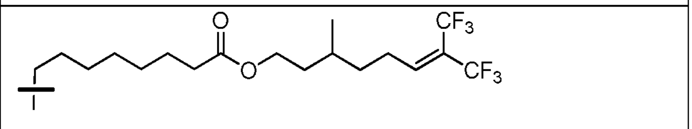
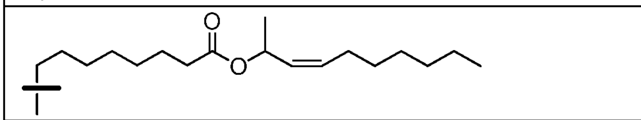
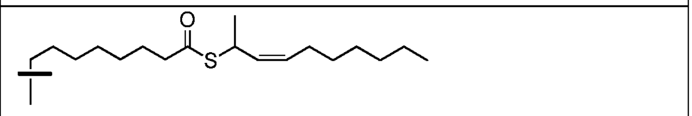
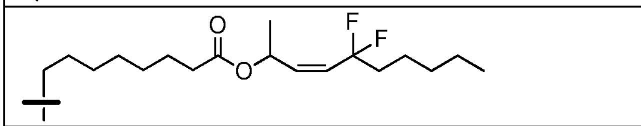
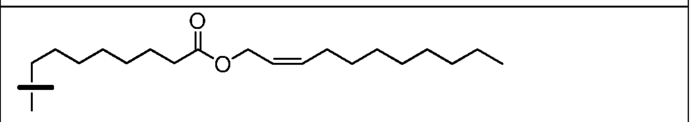
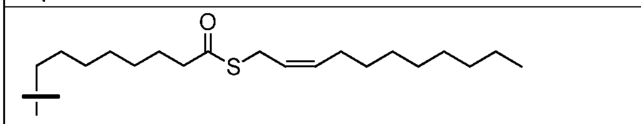
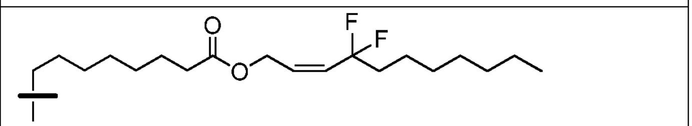
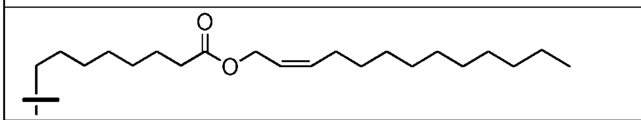
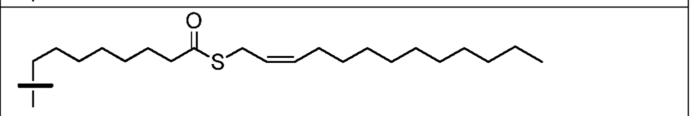
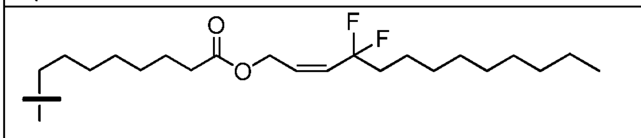
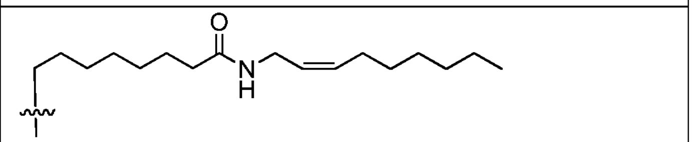
Table 1B

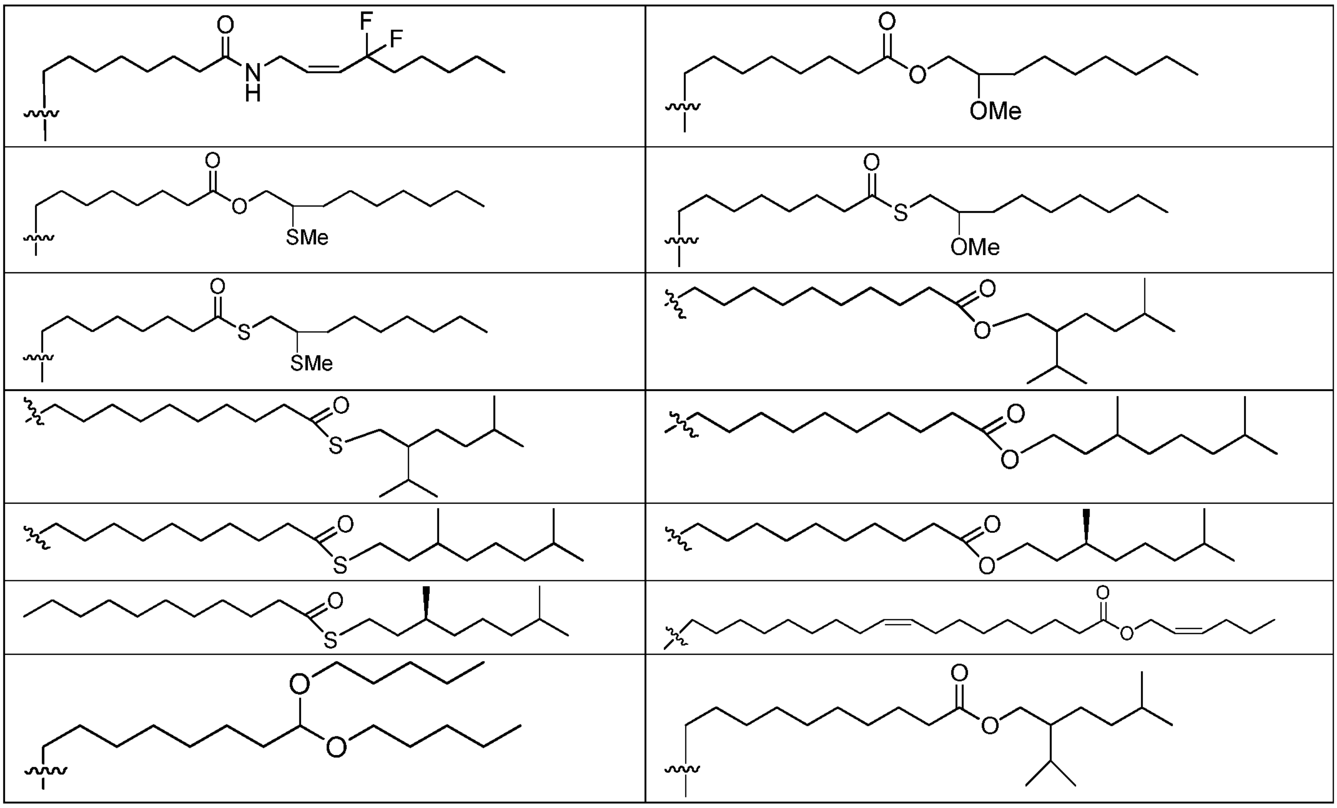
		
		
		

Some suitable hydrophobic tail groups include those depicted in Table 1C:

Table 1C

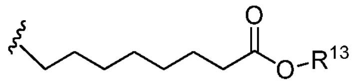


Other suitable tail groups includes those of the formula $–R^{12}-M^1-R^{13}$ where R^{12} is a C_4-C_{14} alkyl or C_4-C_{14} alkenyl, M^1 is a biodegradable group as defined above, and R^{13} is a branched alkyl or alkenyl (e.g., a $C_{10}-C_{20}$ alkyl or $C_{10}-C_{20}$ alkenyl), such that (i) the chain length of $–R^{12}-M^1-R^{13}$ is at most 21 atoms (i.e., the total length of the tail from the first carbon after the tertiary carbon (marked with an asterisk) to a terminus of the tail is at most 21), and (ii) the group $–R^{12}-M^1-R^{13}$ has at least 20 carbon atoms (e.g., at least 21 or 22 carbon atoms).

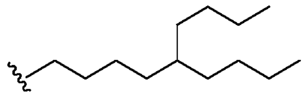
In one preferred embodiment, the chain length of $–R^{12}-M^1-R^{13}$ is at most 21 (e.g., at most 20). For example, the chain length can be from about 17 to about 24 or from about 18 to about 20.

In one embodiment, the total carbon atom content of each tail ($–R^{12}-M^1-R^{13}$) is from about 17 to about 26. For example, the total carbon atom content can be from about 19 to about 26 or from about 21 to about 26.

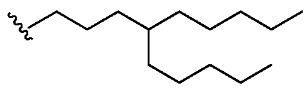
In one embodiment, the tail has the formula:



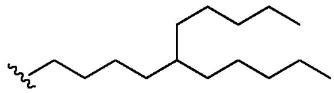
where R^{13} is an alkyl or alkenyl group having from about 13 to about 17 carbon atoms, and the total carbon length of the tail from the first carbon (the leftmost carbon atom above) to a terminus of the tail is at most 20. Preferably, the tail has from about 22 to about 26 carbon atoms. In one embodiment, the maximum length of R^{13} from its attachment point to the ester group of the compound is 12 carbon atoms (e.g., the maximum length can be 11 carbon atoms). In one preferred embodiment, the branch in the alkyl or alkenyl group is at the δ-position or later from the point of attachment of R^{13} to the ester group. Suitable R^{13} groups include, but are not limited to



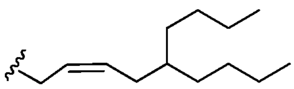
C13 (C21)
Length: C9 (18)



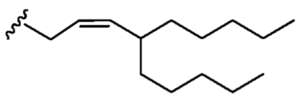
C14 (C22)
Length: C9 (18)



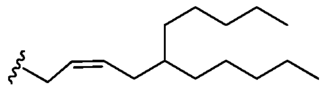
C15 (C23)
Length: C10 (19)



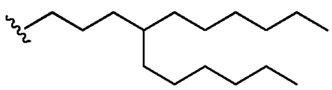
C13 (C21)
Length: C9 (18)



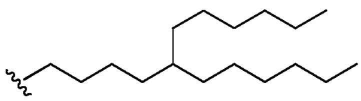
C14 (C22)
Length: C9 (18)



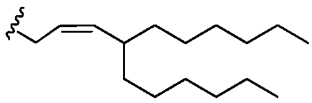
C15 (C23)
Length: C10 (19)



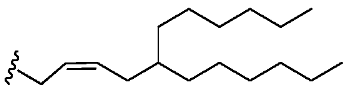
C16 (C24)
Length: C10 (19)



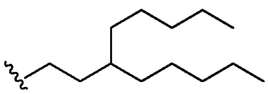
C17 (C25)
Length: C11 (20)



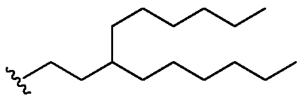
C16 (C24)
Length: C10 (19)



C17 (C25)
Length: C11 (20)

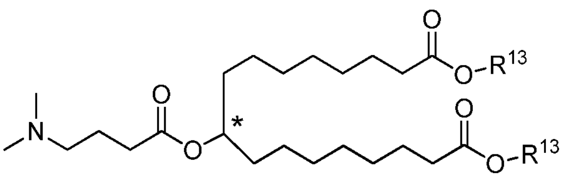


C13 (C21)
Length: C8 (17)



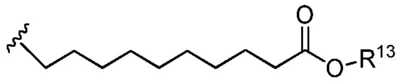
C15 (C23)
Length: C9 (18)

For example, the cationic lipid can be

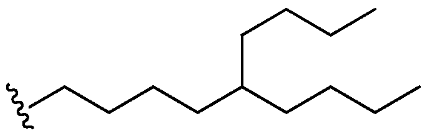


or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), where R^{13} is selected from the groups mentioned above.

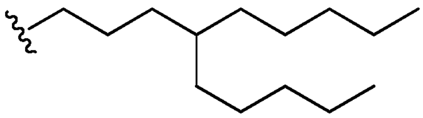
Another example is a tail of the formula



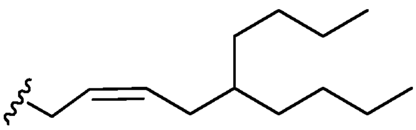
where R^{13} is an alkyl or alkenyl group having from about 13 to about 15 carbon atoms, and the total carbon length of the tail from the first carbon (i.e., the leftmost carbon atom, which is attached to a tertiary carbon) to a terminus of the tail is at most 20. Preferably, the tail has from about 24 to about 26 carbon atoms. In one embodiment, the maximum length of R^{13} from its attachment point to the ester group of the compound is 10 carbon atoms (e.g., the maximum length can be 9 carbon atoms). In one preferred embodiment, the branch in the alkyl or alkenyl group is at the δ-position or later from the point of attachment of R^{13} to the ester group. Suitable R^{13} groups include, but are not limited to



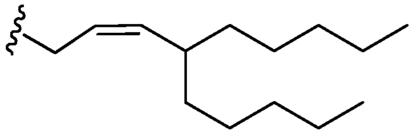
C13 (C23)
Length: C9 (20)



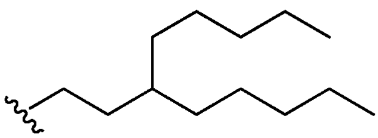
C14 (C24)
Length: C9 (20)



C13 (C23)
Length: C9 (20)

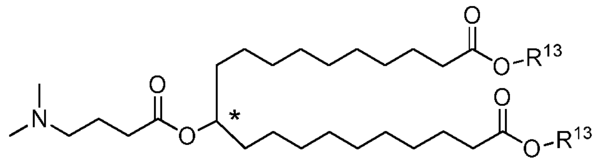


C14 (C24)
Length: C9 (20)



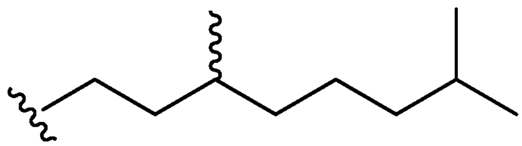
C13 (C24)
Length: C8 (19)

For example, the cationic lipid can be

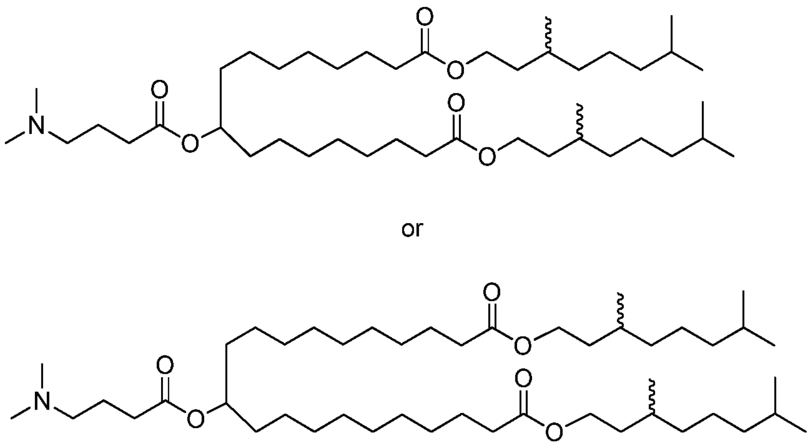


or a salt thereof (e.g., a pharmaceutically acceptable salt thereof), where R^{13} is selected from the groups above.

The R^{13} group may be derived from a natural product, such as dihydrocitgronellol, lavandulol, phytol, or dihydrophytol. In one embodiment, the R^{13} group in the tails above is a dihydrocitronellol group (either as a racemic group or a chirally pure group):

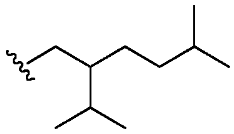
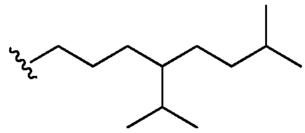


For example, the cationic lipid having a dihydroitronellol group can be

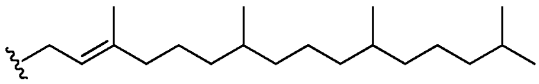


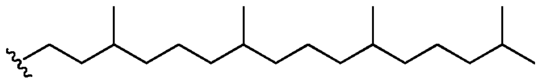
or a salt thereof.

In another embodiment, the R^{13} group in the tails above is a lavandulol group or a homolog of it as shown below:

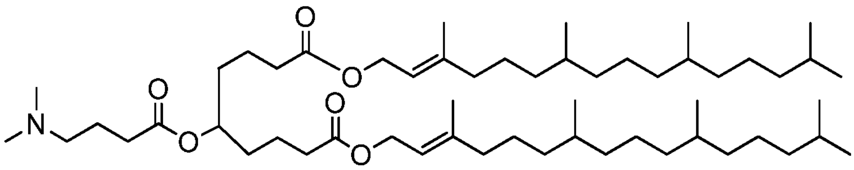
 Lavandulol  homolog

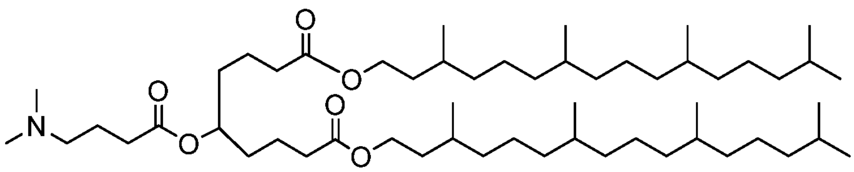
In another embodiment, the R^{13} group in the tails above is a phytol or dihydrophytol group:

 Phytol

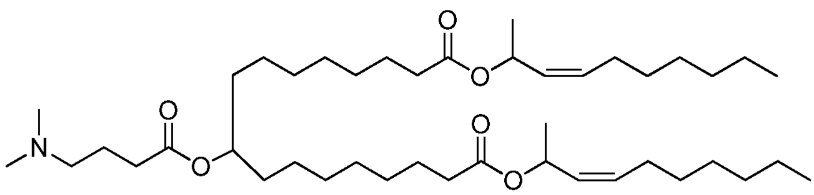
 Dihydrophytol

For instance, the cationic lipid can be:

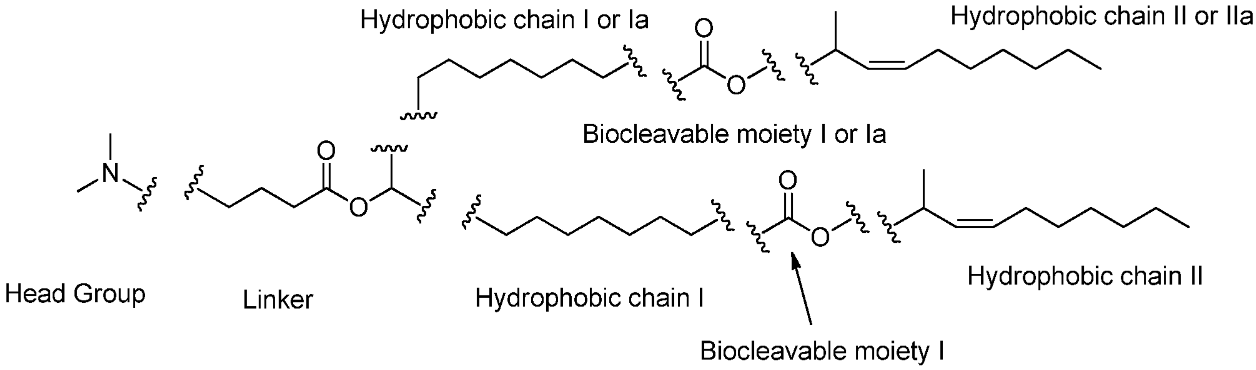




A cationic lipid of the fomula:

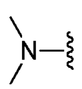
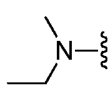
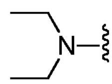
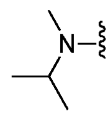
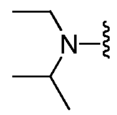
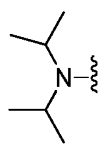
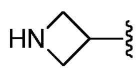
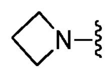
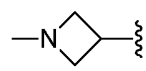
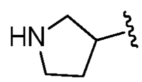
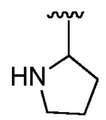
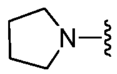
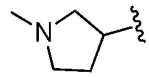
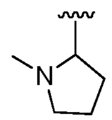
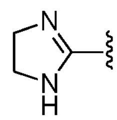
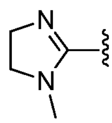
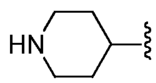
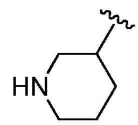
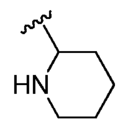
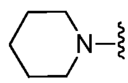
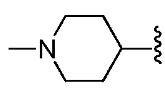
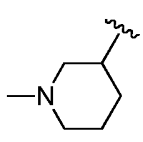
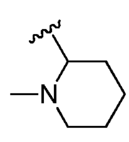
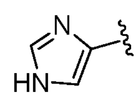
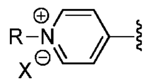
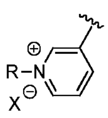
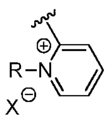
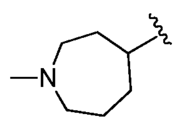
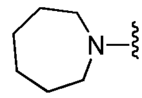
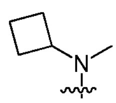


can also be thought of as a combination of a headgroup, a linker moiety, and two parts of the hydrophobic chains as follows:



Various headgrops, linker moieties, and hydrophobic chains I and II are listed below. The present invention includes compounds composed of any combination of the head, linker, hydrophobic chain I, and hydrophobic chain II groups listed below.

Table 2A - Representative headgroups

				
				
				
				
				 R = H, alkyl; X = halogen
 R = H, alkyl; X = halogen	 R = H, alkyl; X = halogen			

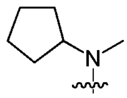
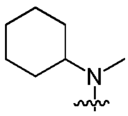
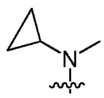
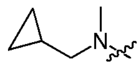
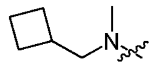
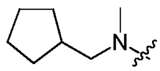
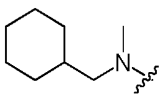
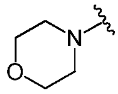
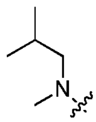
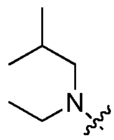
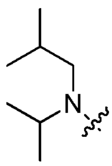
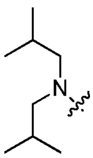
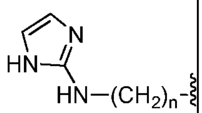
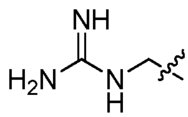
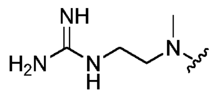
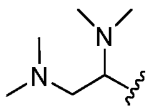
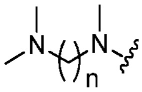
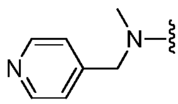
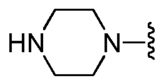
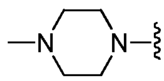
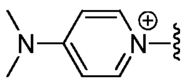
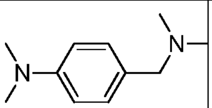
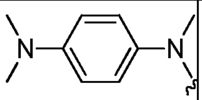
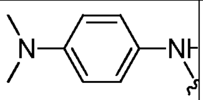
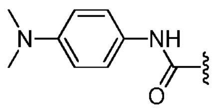
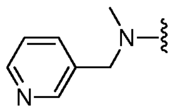
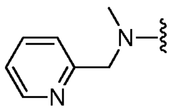
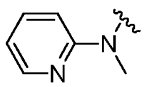
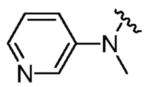
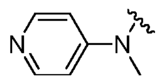
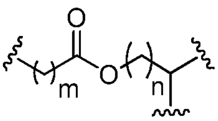
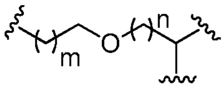
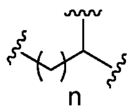
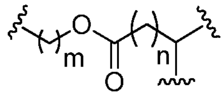
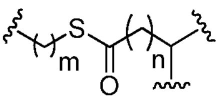
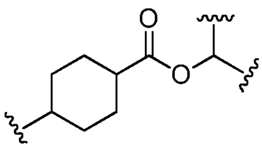
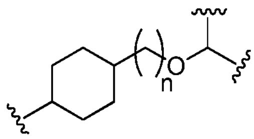
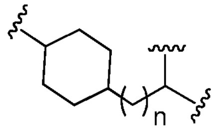
				
				
		 (where n is 0-5)		
	 n = 0-6			
				
				

Table 2B - Representative linker groups

 m = 1-5; n = 0-3	 m = 0-5, n = 0-3	 n = 0-5	 m = 0-5; n = 0-3
 m = 0-5; n = 0-3		 n = 0-3	 n = 0-3

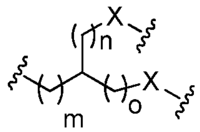
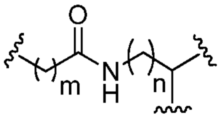
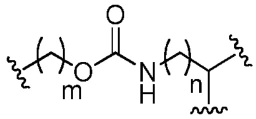
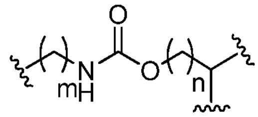
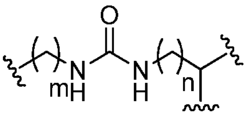
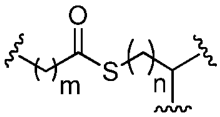
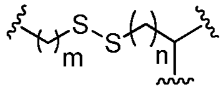
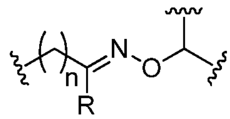
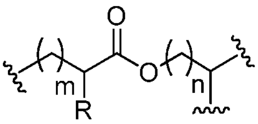
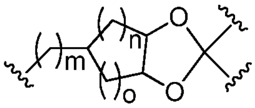
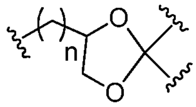
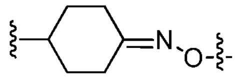
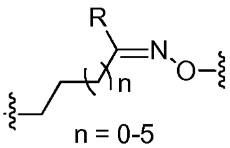
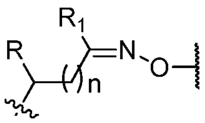
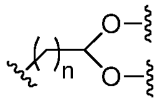
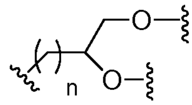
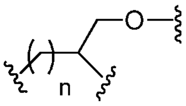
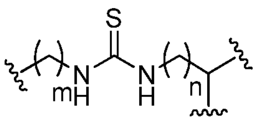
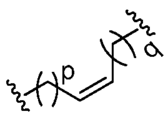
 m = 1-4; n/o = 0-3 x = O or S	 m = 0-5; n = 0-3	 m = 0-5; n = 0-3	 m = 0-5; n = 0-3
 m = 0-5; n = 0-3	 m = 0-5; n = 0-3	 m = 0-5; n = 0-3	 n = 0-5
 m = 1-4; n = 0-3 R = COOH, COOMe, COOEt, CN, CONH2 CONHMe	 m = 1-4; n/o = 1-3	 n = 1-5	
 n = 0-5 R = H, Me, Et, Pr, allyl	 n = 0-5 R = Me, Et, Pr, allyl R1= Me, Et, Pr, allyl	 n = 0-6	 n = 0-6
 n = 0-6	 m = 0-5; n = 0-3		

Table 2C - Representative hydrophobic chain I and/or Ia, and combination thereof

 p = 0-15	 p = 0-15, q = 0-15

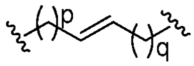
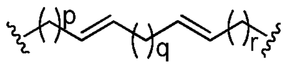
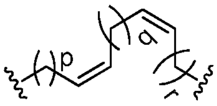
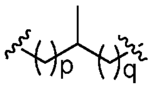
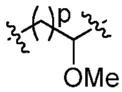
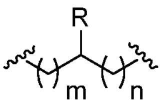
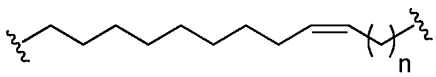
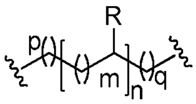
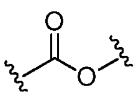
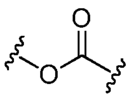
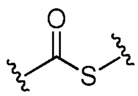
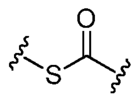
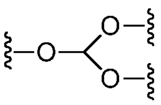
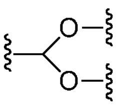
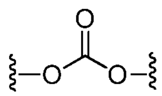
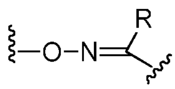
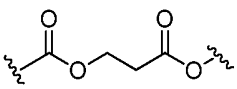
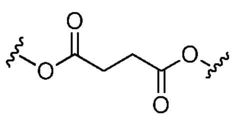
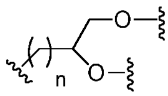
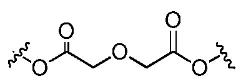
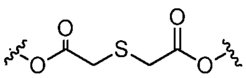
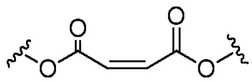
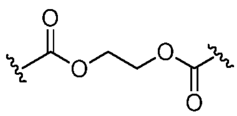
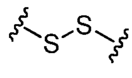
 p = 0-15, q = 0-15	 p = 0-15, q = 1-4, r = 0-15
 p = 0-15, q = 1-4, r = 0-15	
 p = 0-15, q = 0-6	 p = 0-15
 m = 0-4; n = 0-4; R = Me, Et, Pr, iPr, Bu, iBu	 n = 1-7
 m = 1-4, n = 1-10, p = 0-15, q = 0-15 R = Me, Et, OMe	

Table 2D - Representative biodegradable moieties I and/or Ia and combinations thereof

			
			
		 n = 0-6	
			

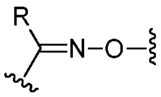
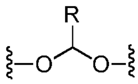
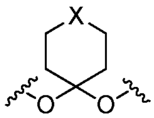
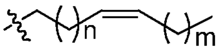
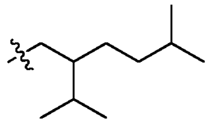
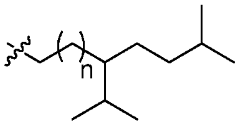
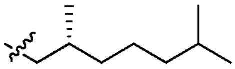
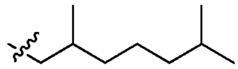
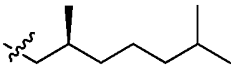
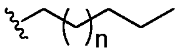
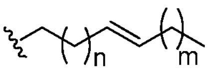
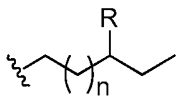
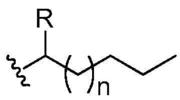
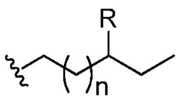
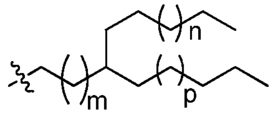
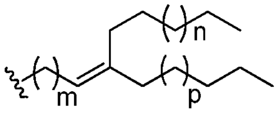
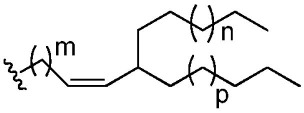
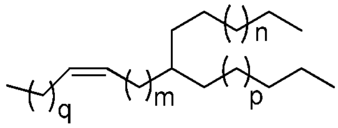
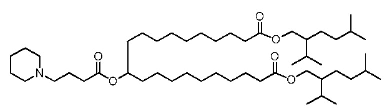
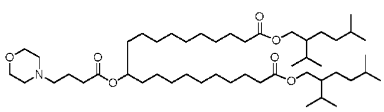
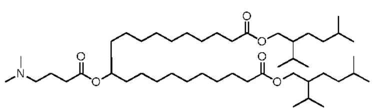
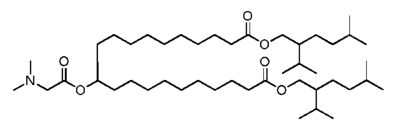
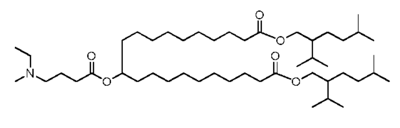
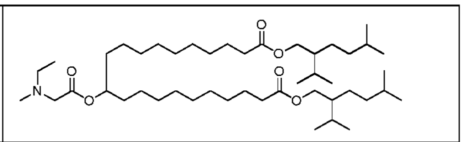
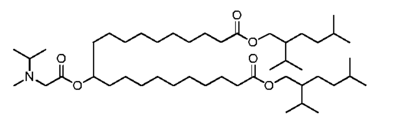
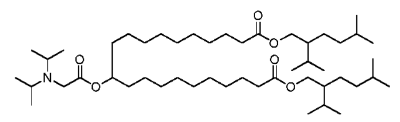
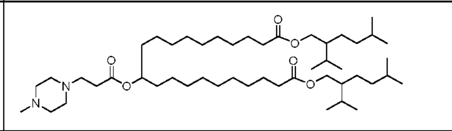
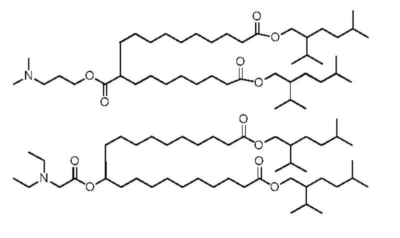
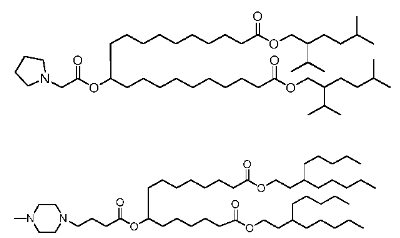
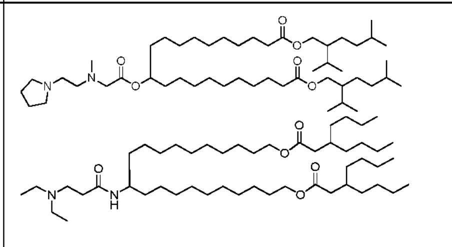
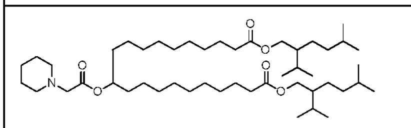
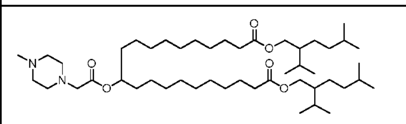
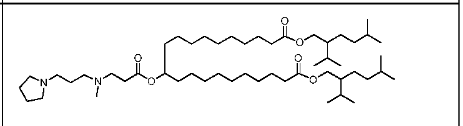
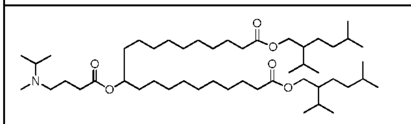
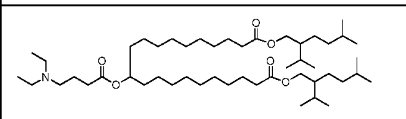
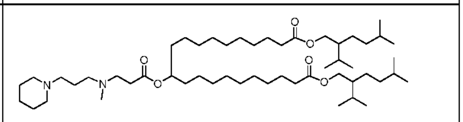
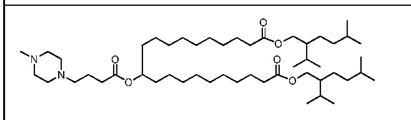
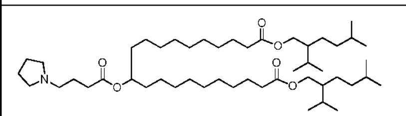
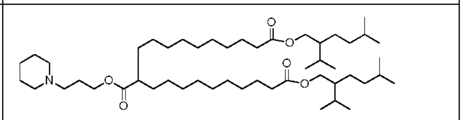
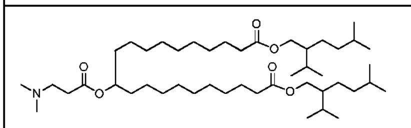
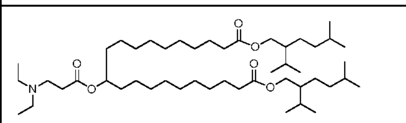
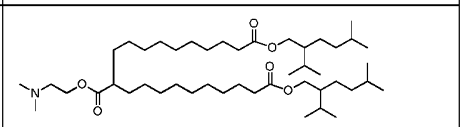
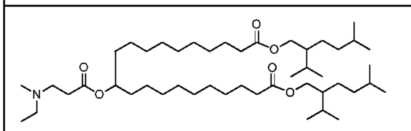
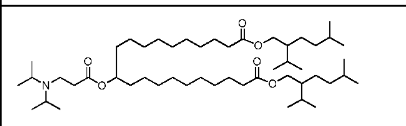
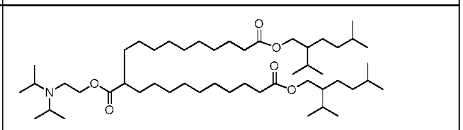
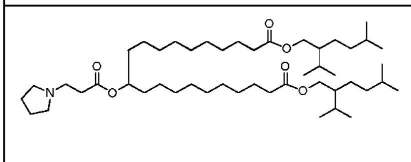
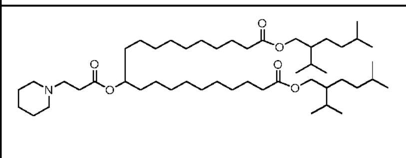
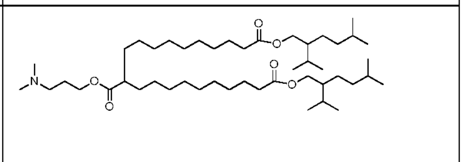
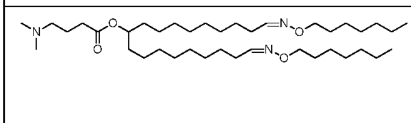
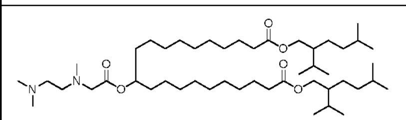
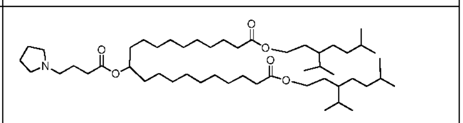
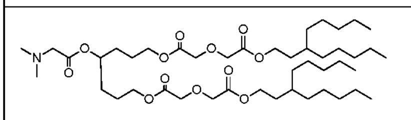
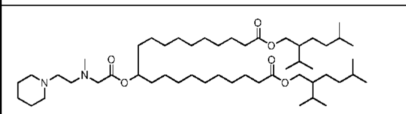
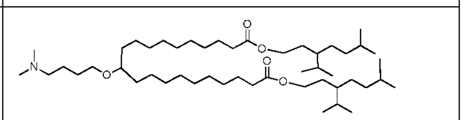
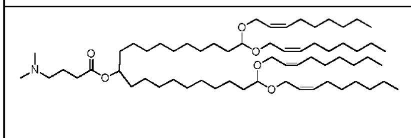
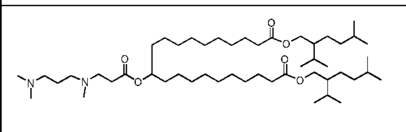
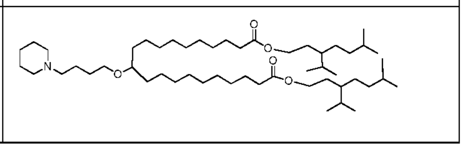
	 R = H, Me, Et, cyclic alkyl, alicylic, aromatic	 X = CH_2, O. S	

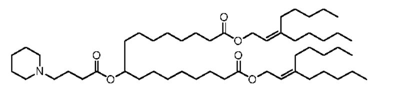
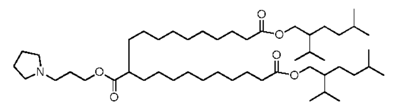
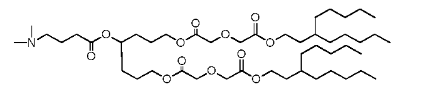
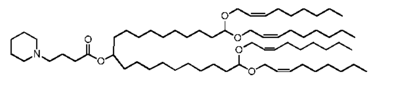
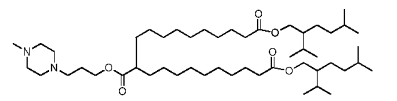
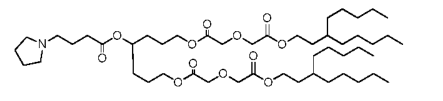
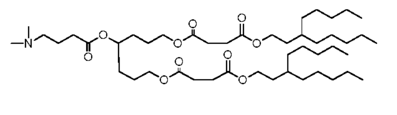
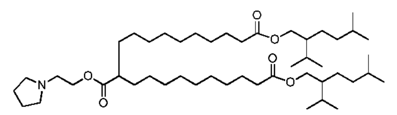
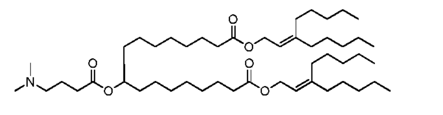
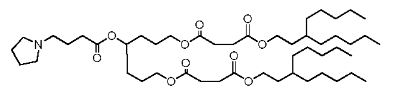
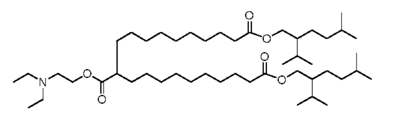
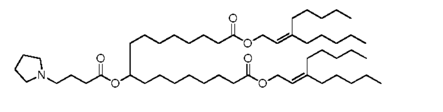
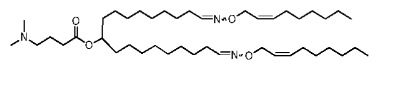
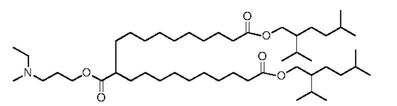
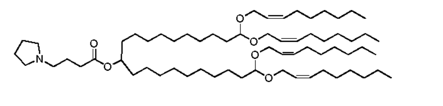
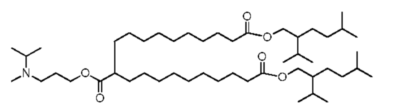
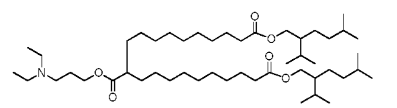
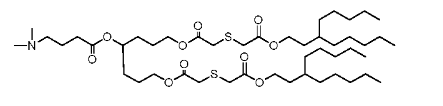
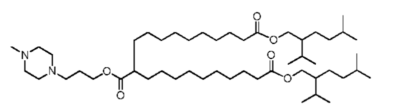
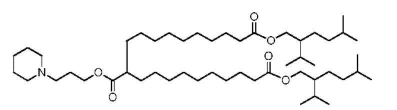
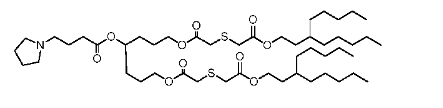
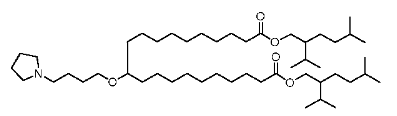
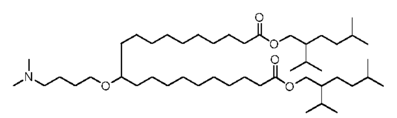
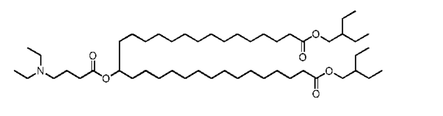
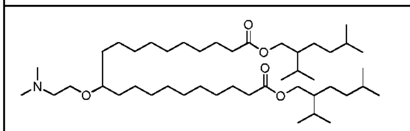
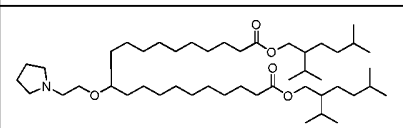
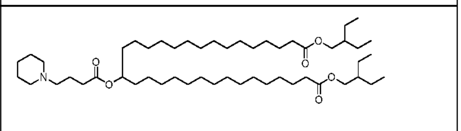
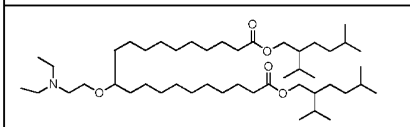
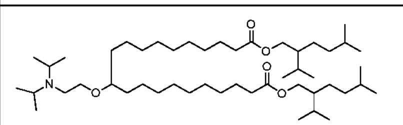
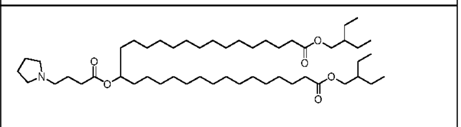
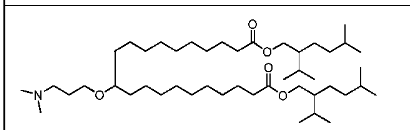
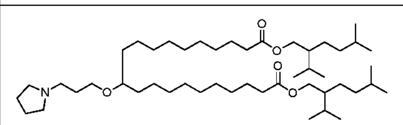
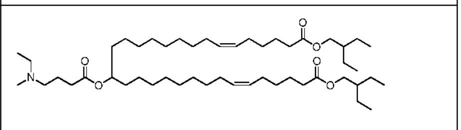
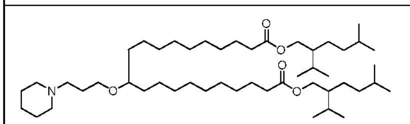
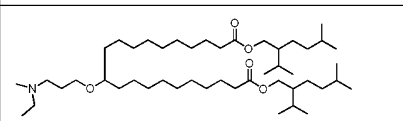
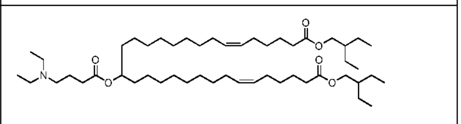
Table 2E - Representative hydrophobic chain II and/or IIa and combinations thereof

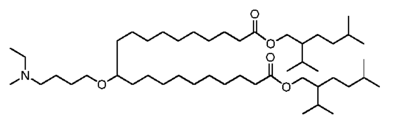
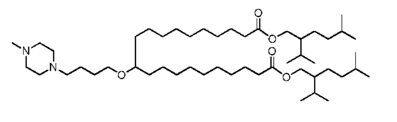
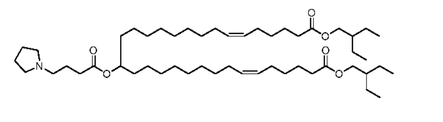
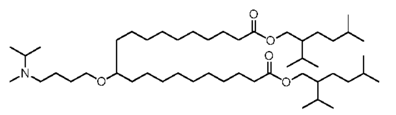
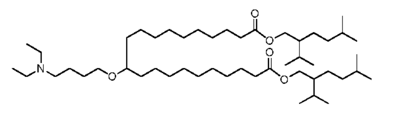
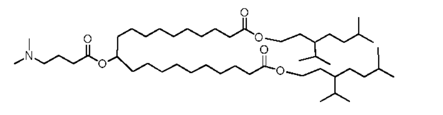
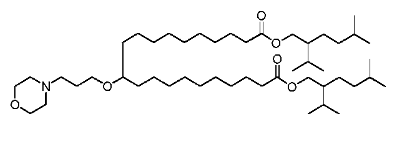
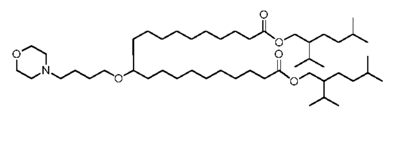
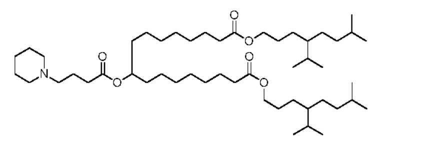
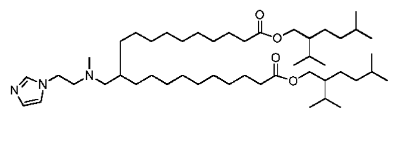
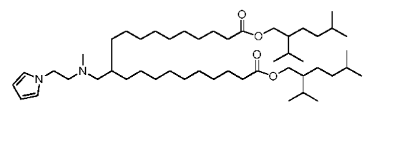
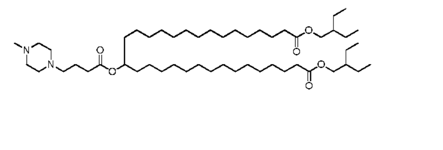
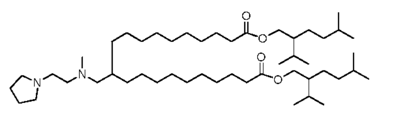
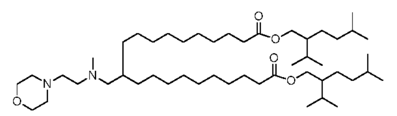
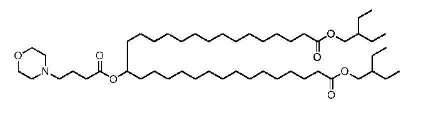
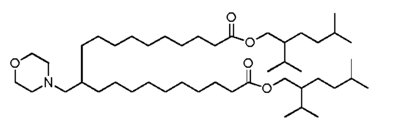
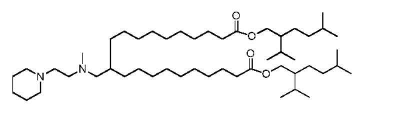
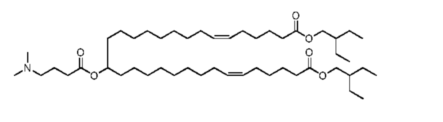
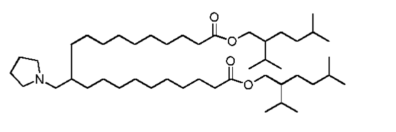
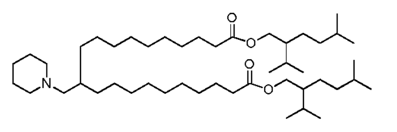
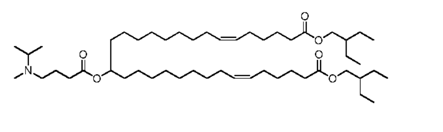
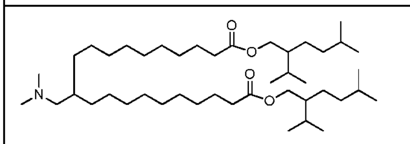
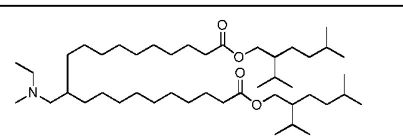
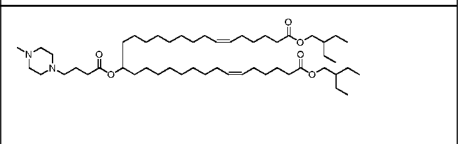
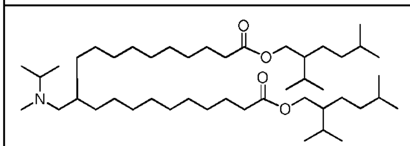
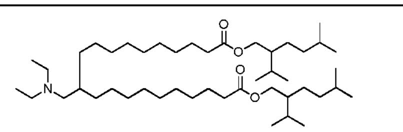
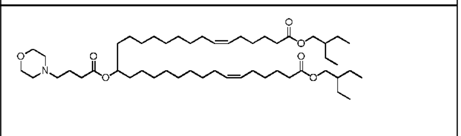
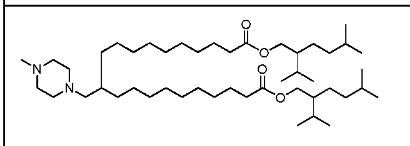
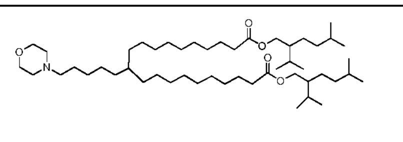
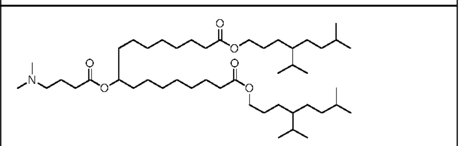
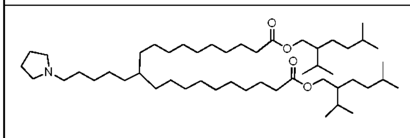
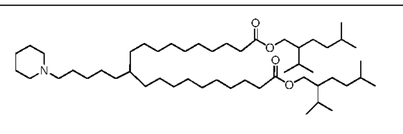
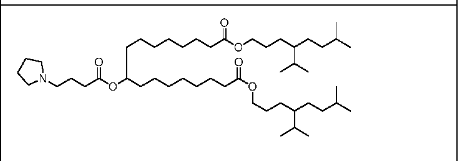
 n = 0-6; m = 0-16		 n = 0-6
		
 n = 0-8	 n = 0-8; m = 0-6	 n = 0-8 R =OMe, Me, Et, n-Pr, n-Bu
 n = 0-8 R =OMe, Me, Et, Pr	 n = 0-8 R =OMe, Me, Et, Pr	 m =0-6; n = 0-6; p = 0-6
 m =0-6; n = 0-6; p = 0-6	 m =0-6; n = 0-6; p = 0-6	 m =0-6; n = 0-6; p = 0-6; q = 0-6

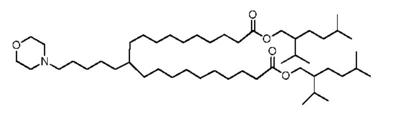
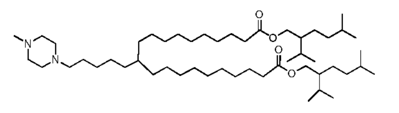
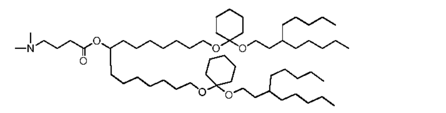
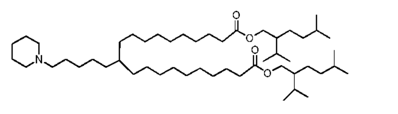
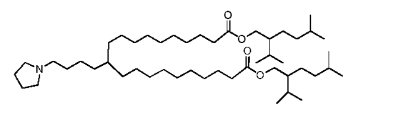
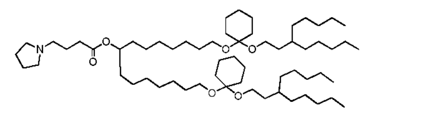
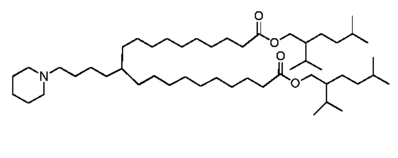
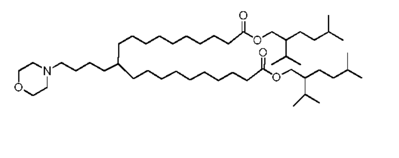
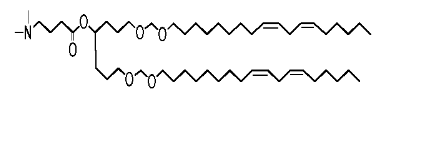
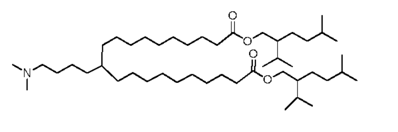
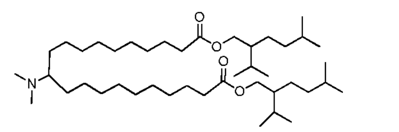
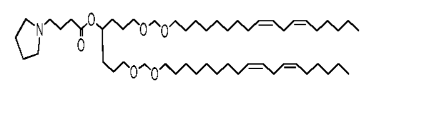
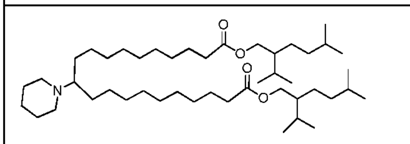
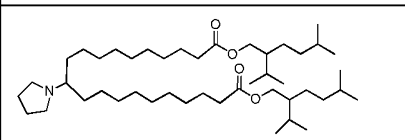
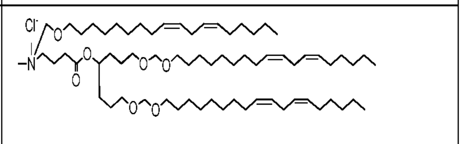
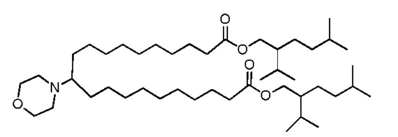
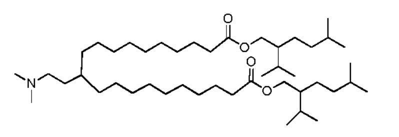
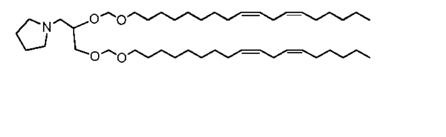
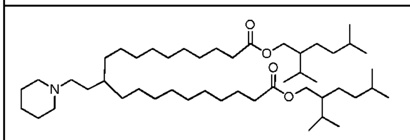
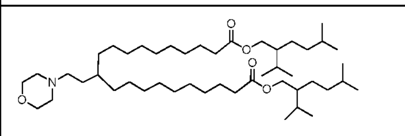
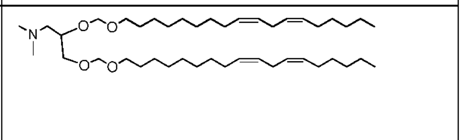
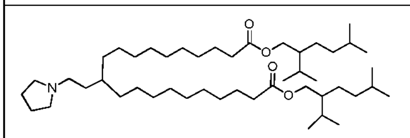
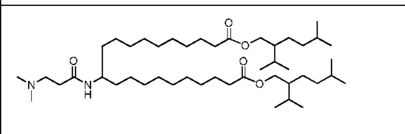
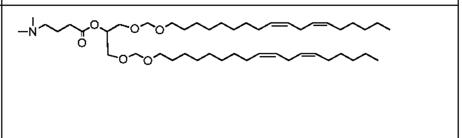
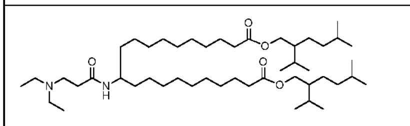
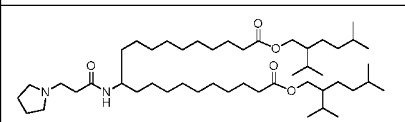
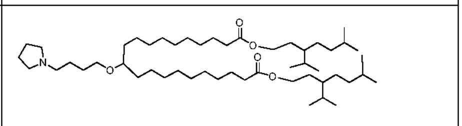
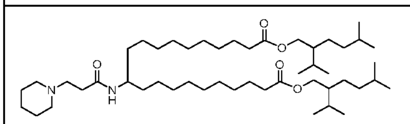
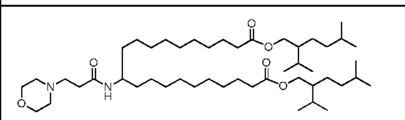
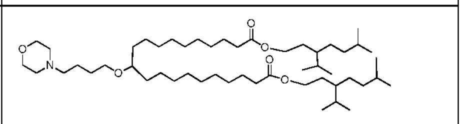
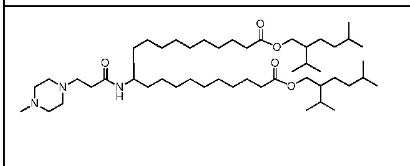
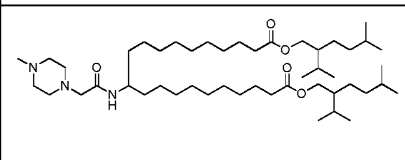
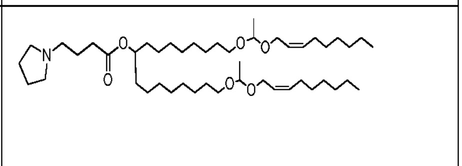
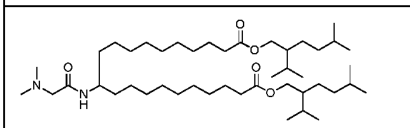
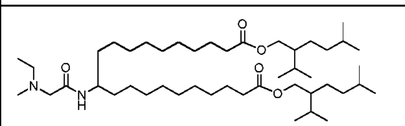
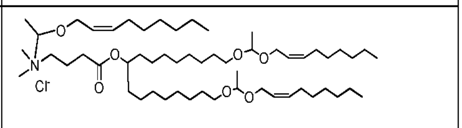
Other cationic lipids of the present invention include those in Table 3 below. Each asymmetric carbon atom in the compounds below can be either chirally pure (*R* or *S*) or racemic. These cationic lipids as well as those in the working examples (such as Examples 36 and 37) are suitable for forming nucleic acid-lipid particles.

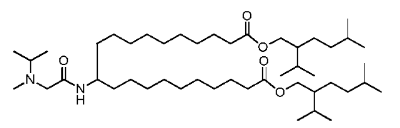
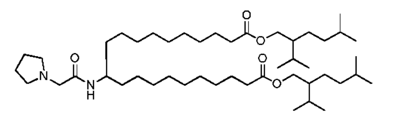
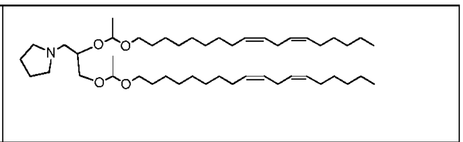
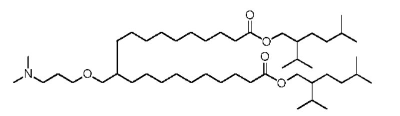
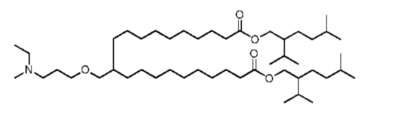
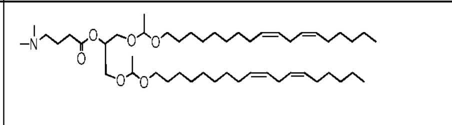
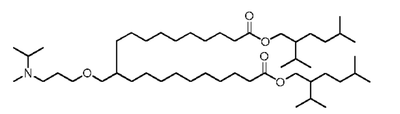
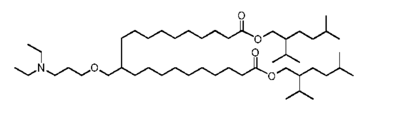
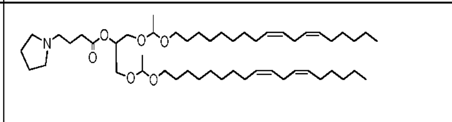
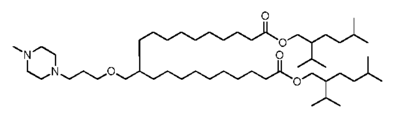
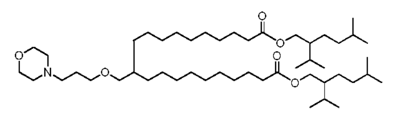
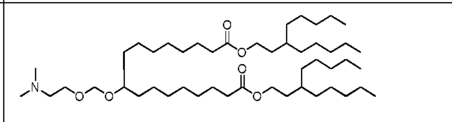
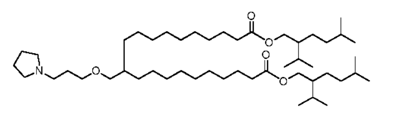
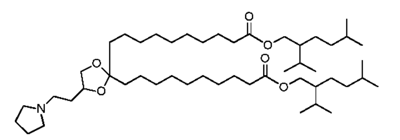
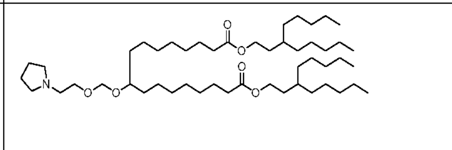
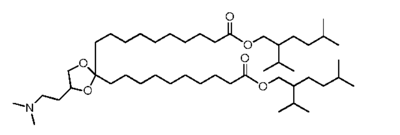
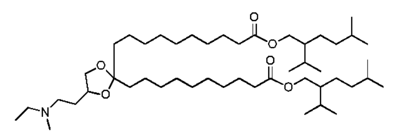
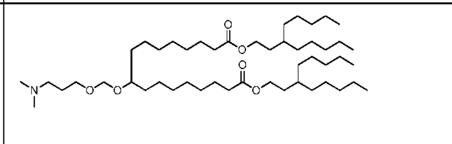
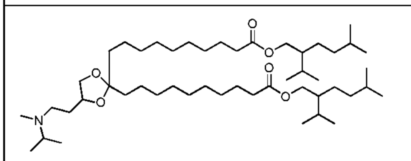
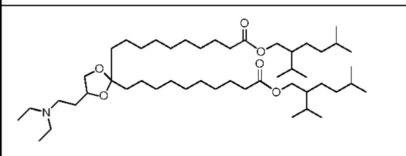
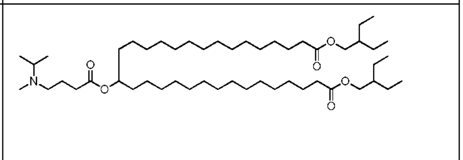
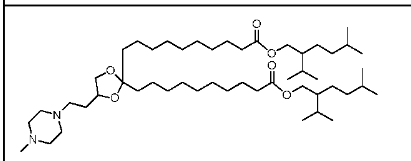
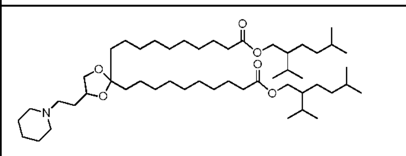
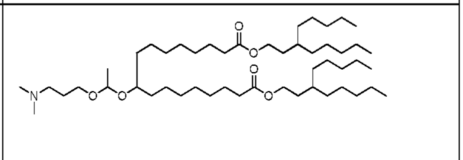
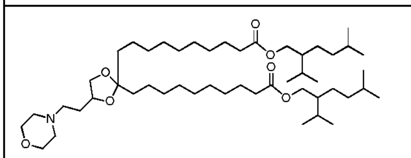
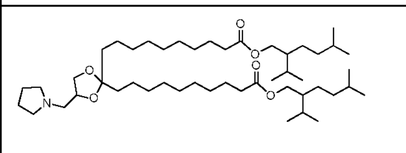
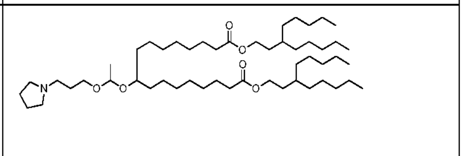
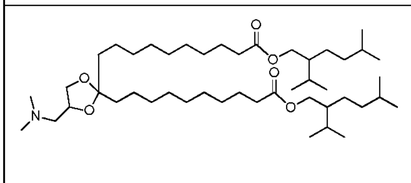
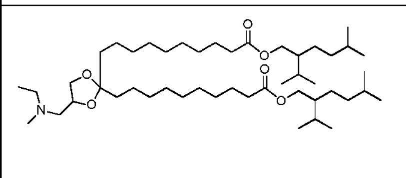
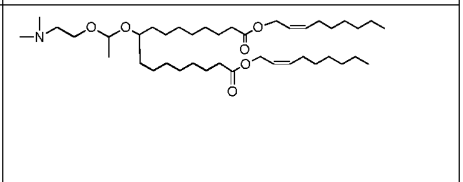
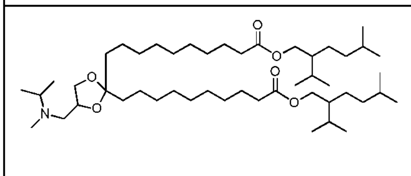
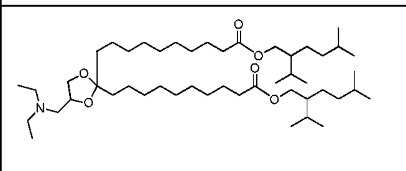
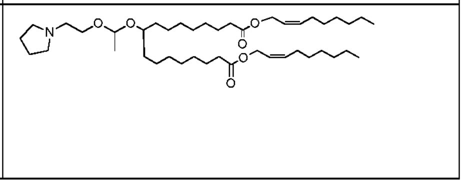
		

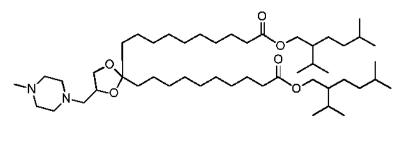
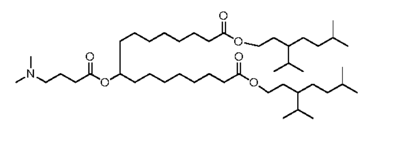
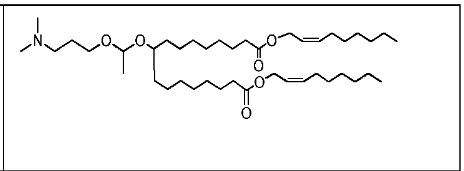
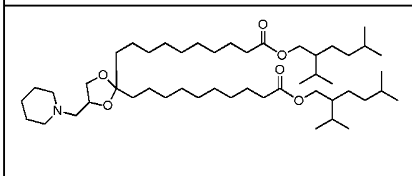
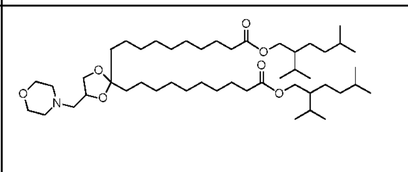
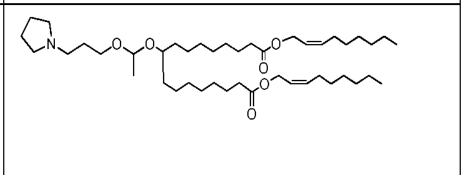
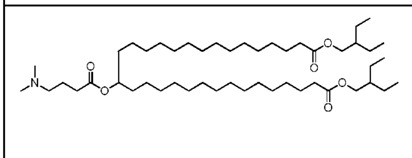
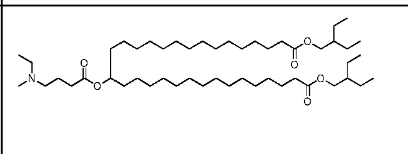
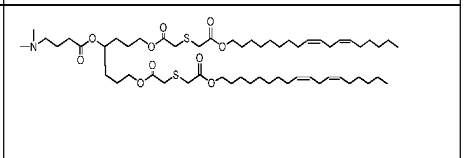
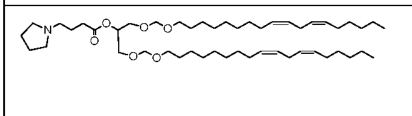
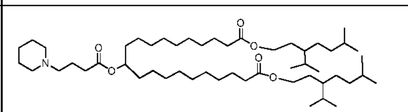
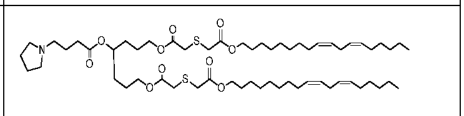
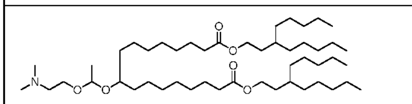
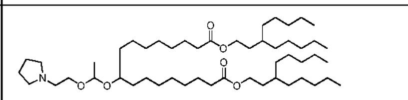
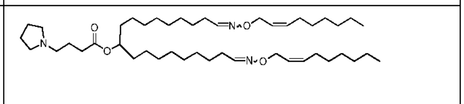
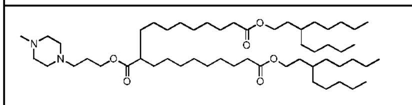
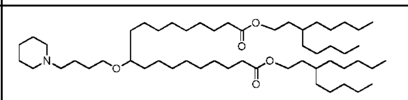
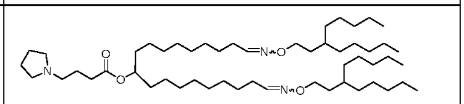
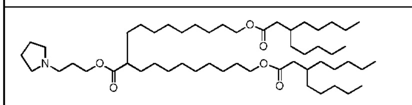
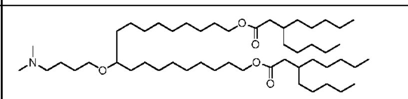
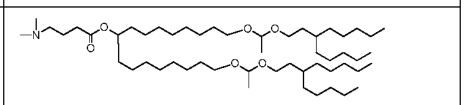
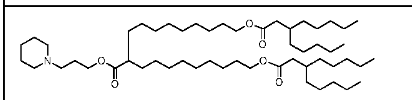
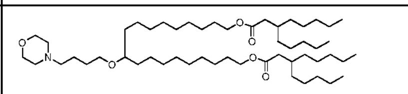
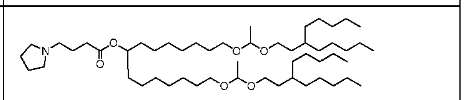
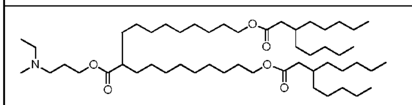
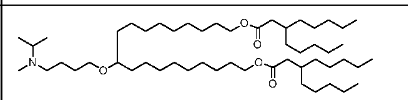
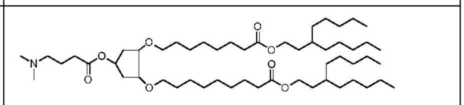
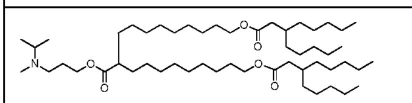
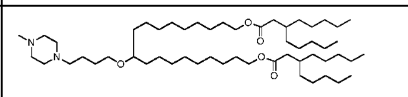
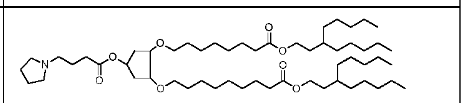
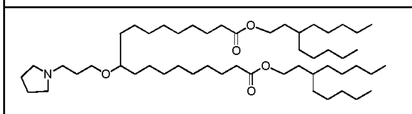
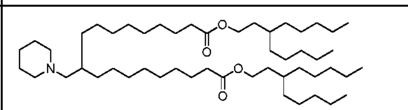
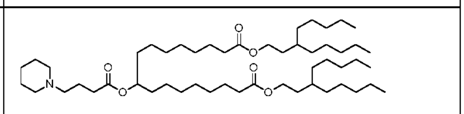
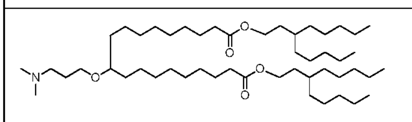
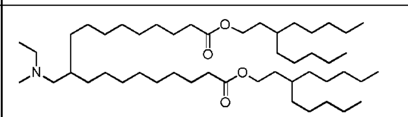
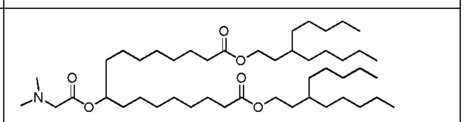
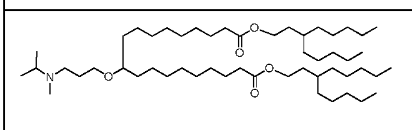
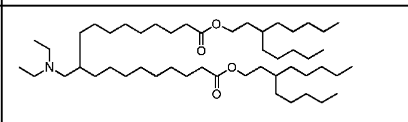
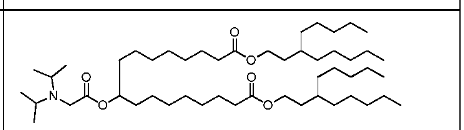
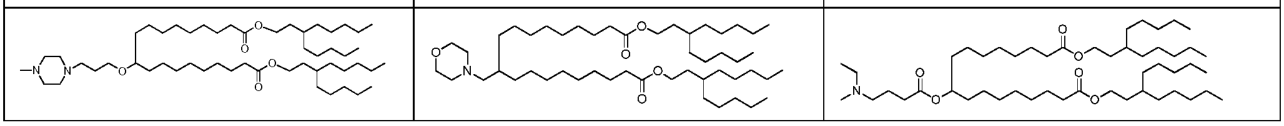
		
		
		
		
		
		
		
		
		
		
		
		

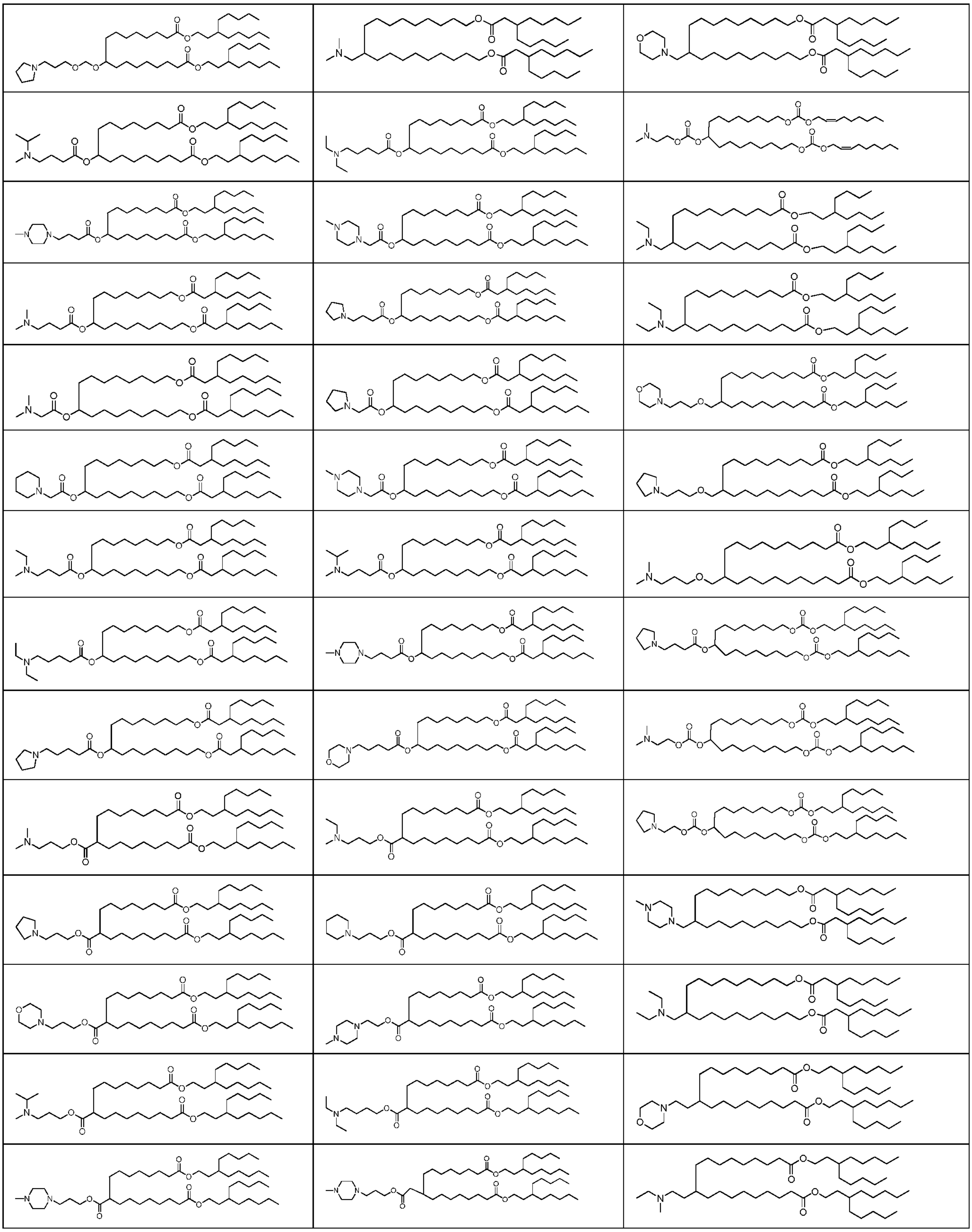
		
		
		
		
		
		
		
		
		
		
		
		

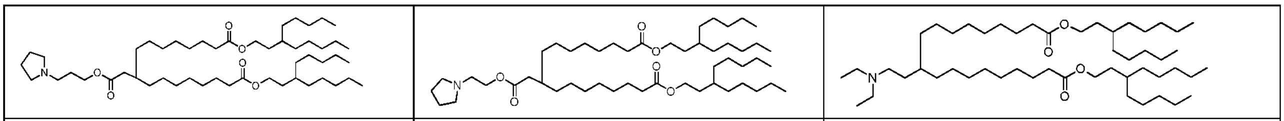
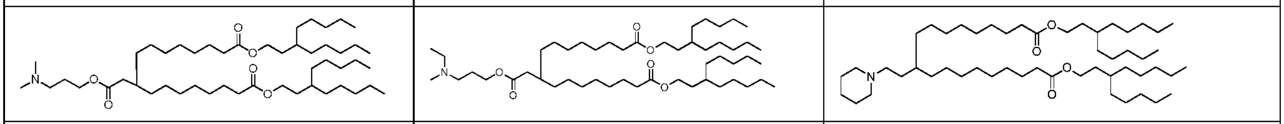
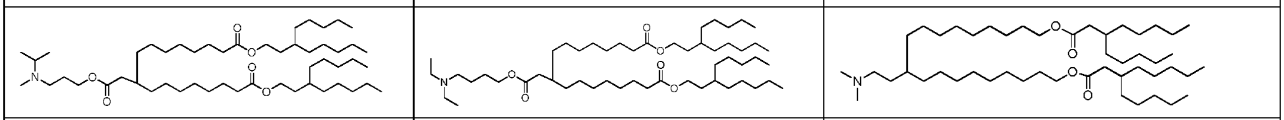
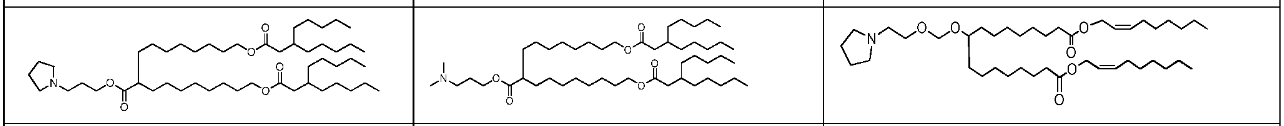
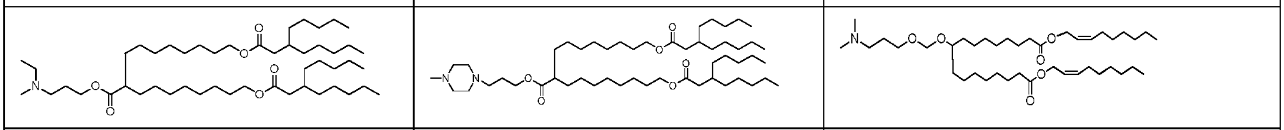
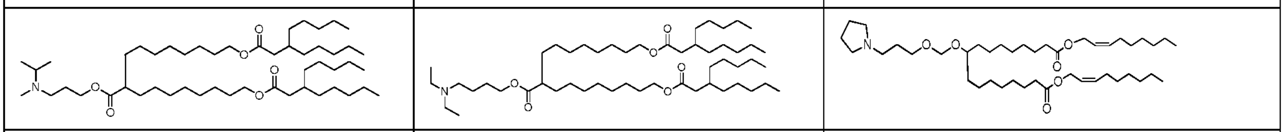
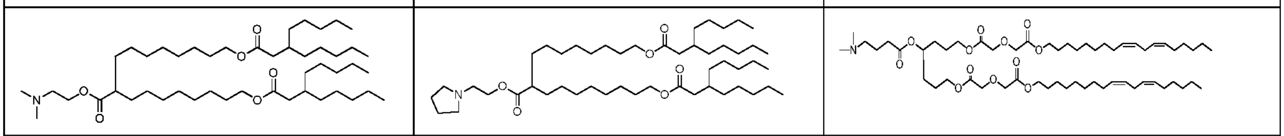
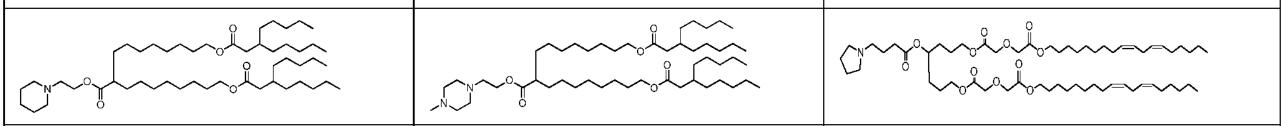
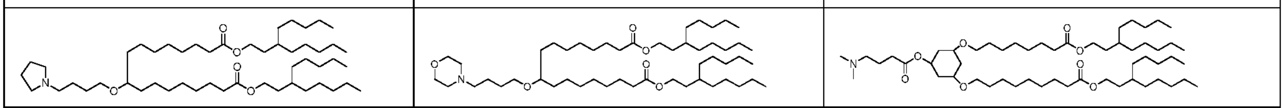
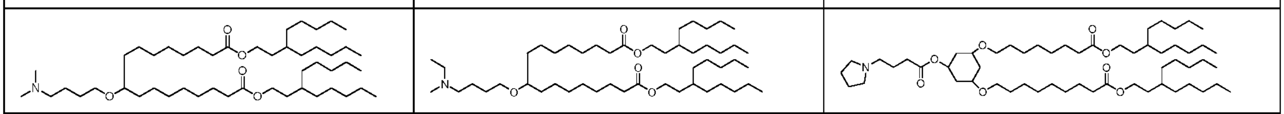
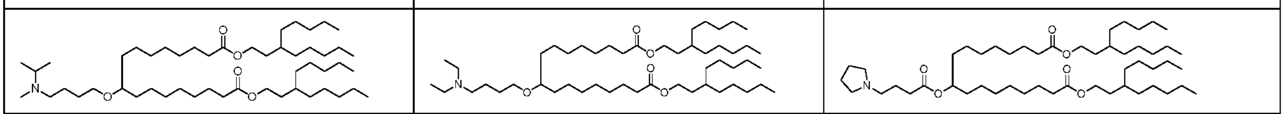
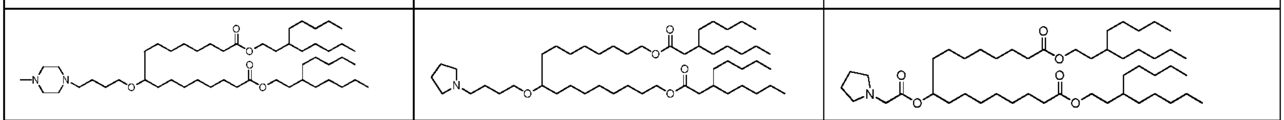
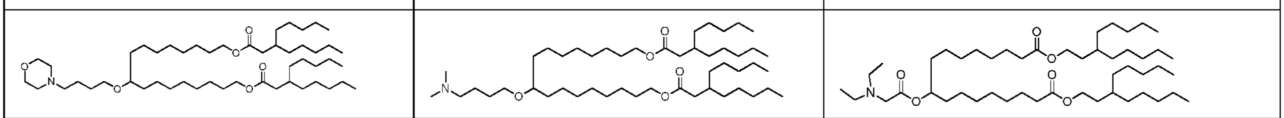
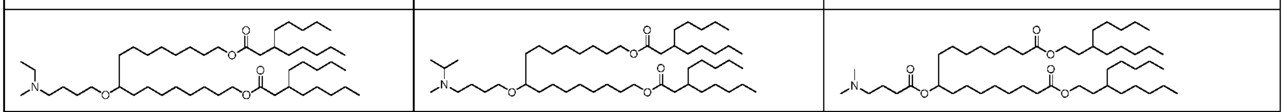
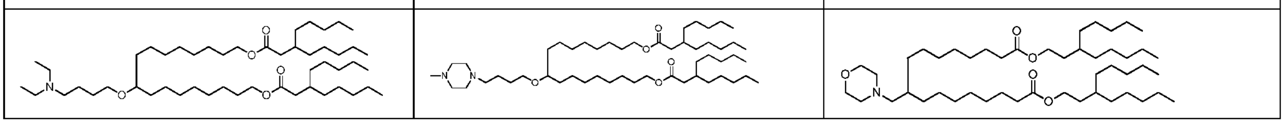
		
		
		
		
		
		
		
		
		
		
		

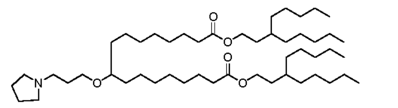
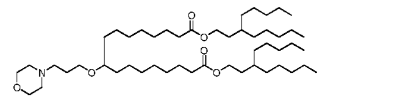
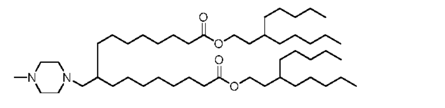
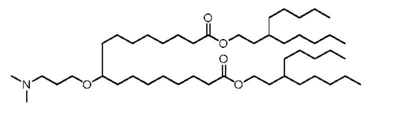
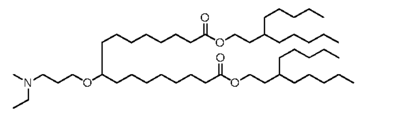
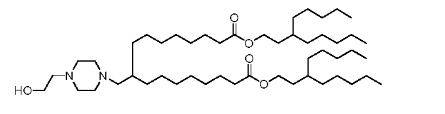
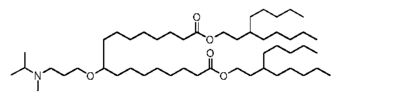
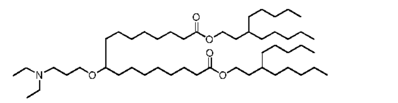
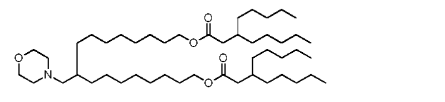
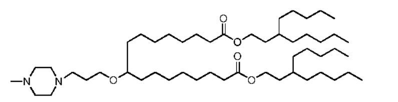
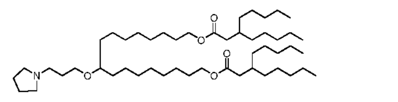
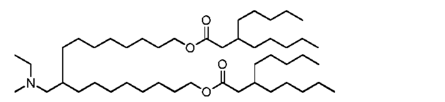
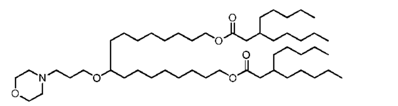
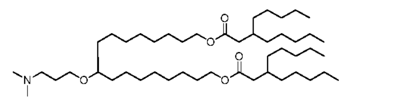
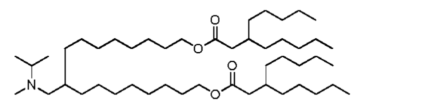
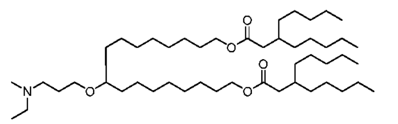
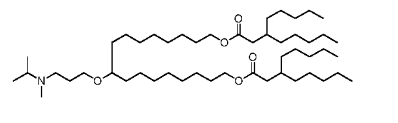
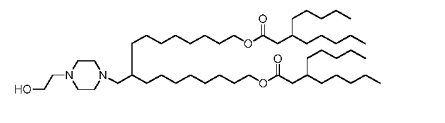
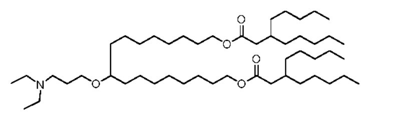
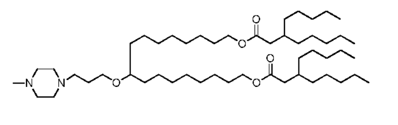
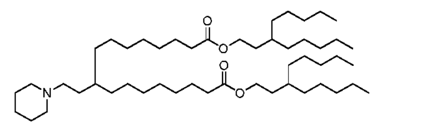
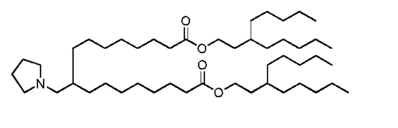
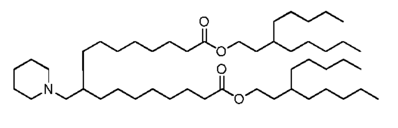
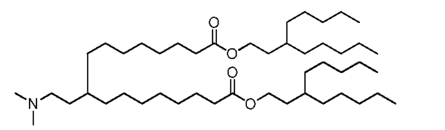
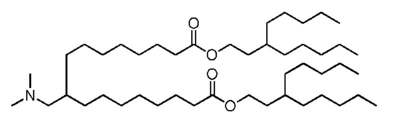
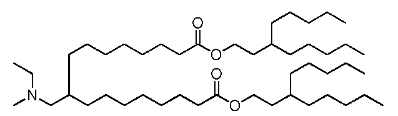
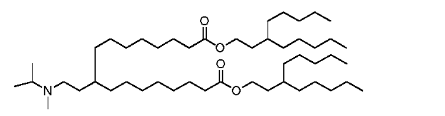
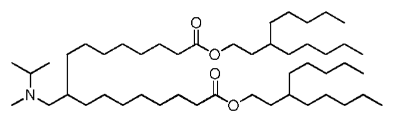
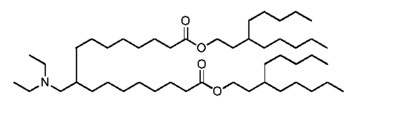
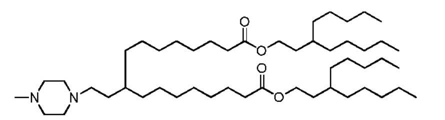
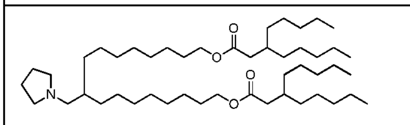
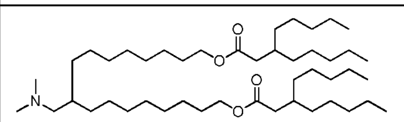
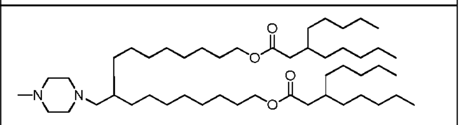
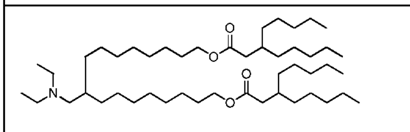
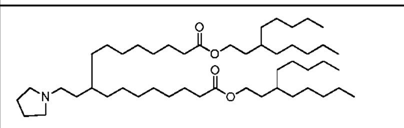
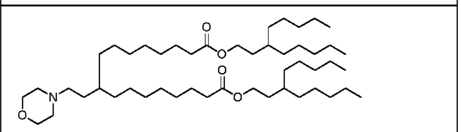
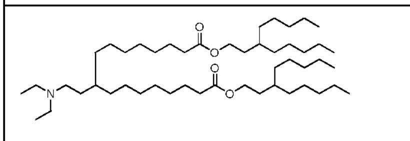
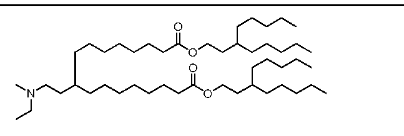
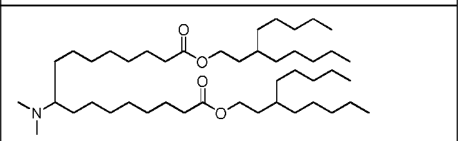
		
		
		
		
		
		
		
		
		
		
		
		

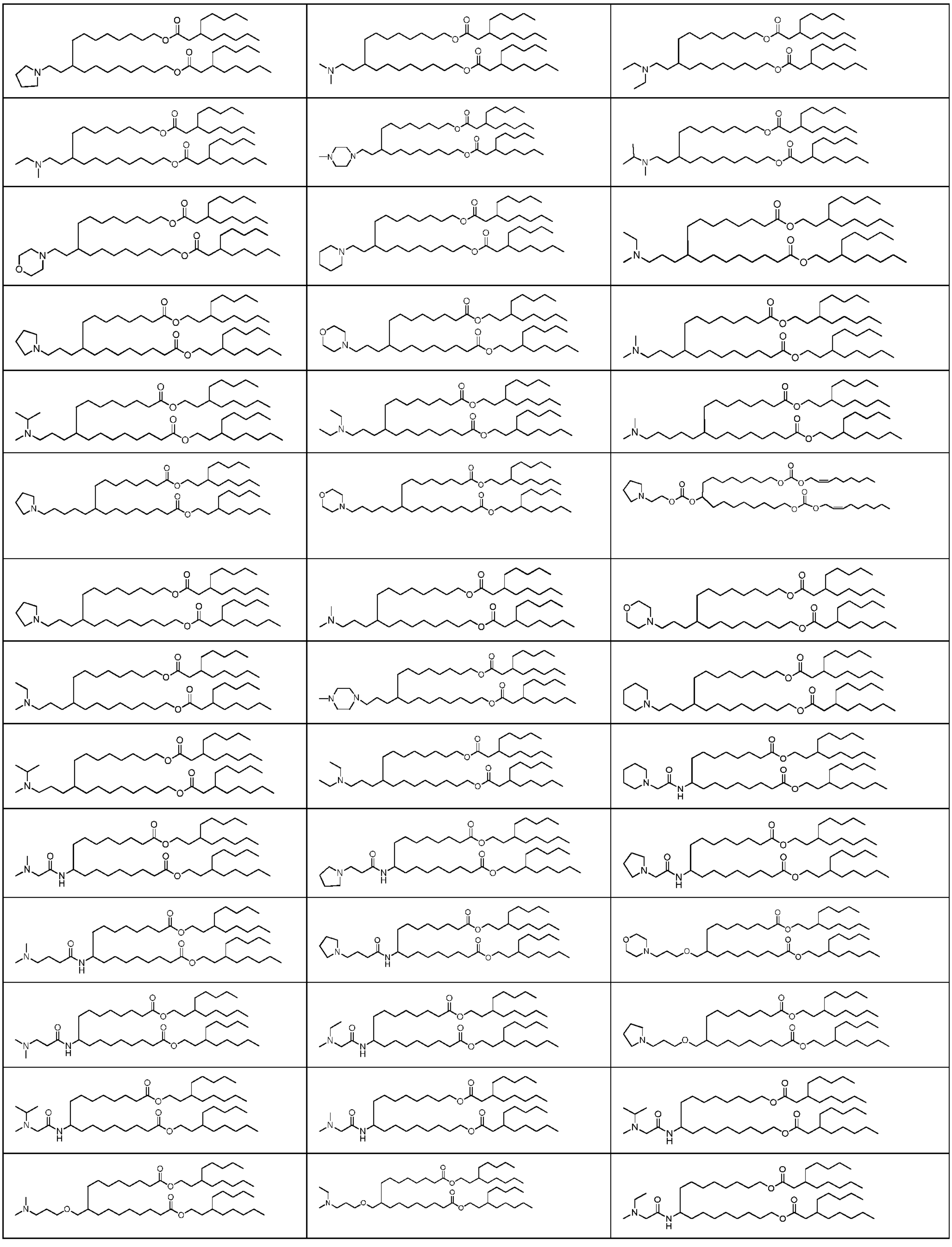
		
		
		
		
		
		
		
		
		
		
		

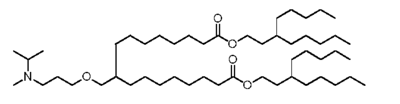
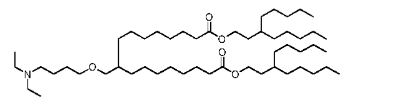
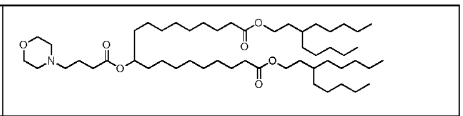
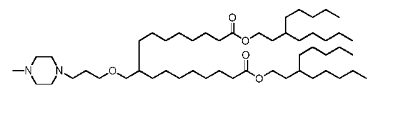
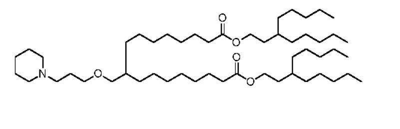
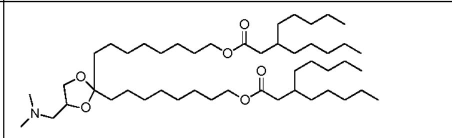
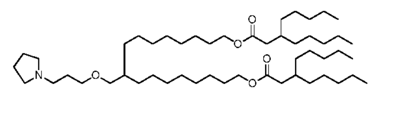
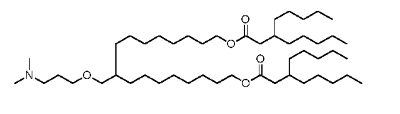
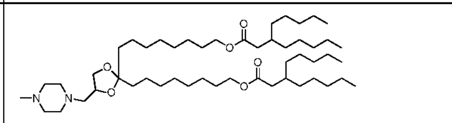
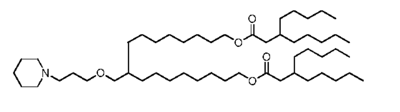
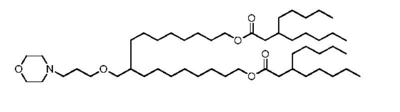
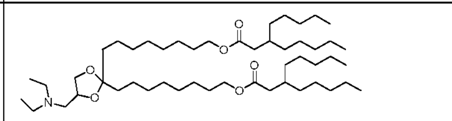
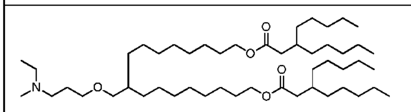
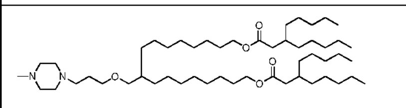
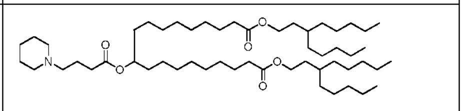
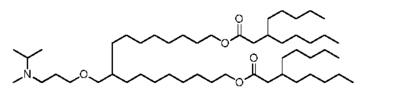
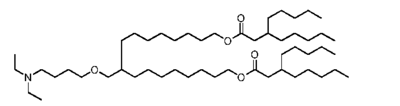
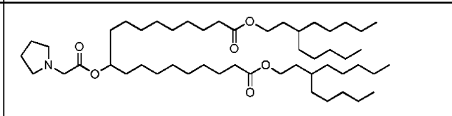
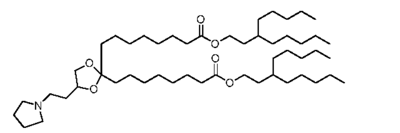
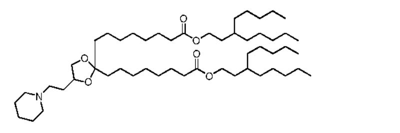
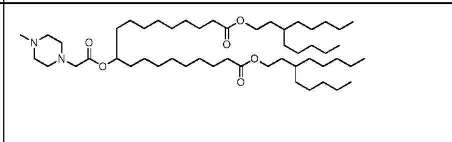
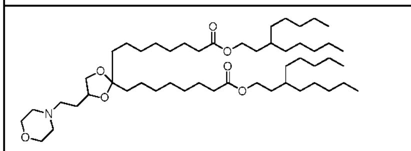
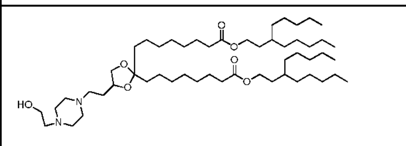
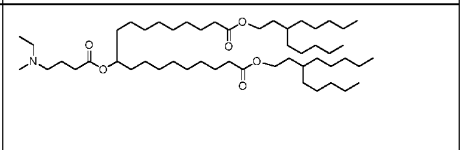
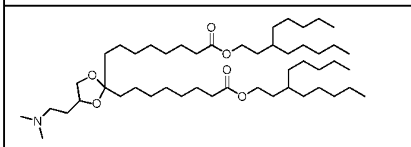
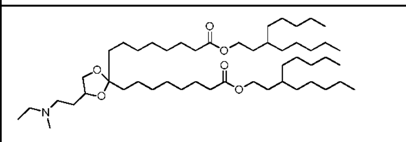
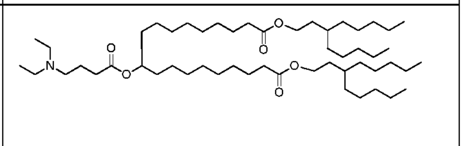
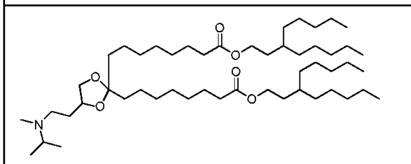
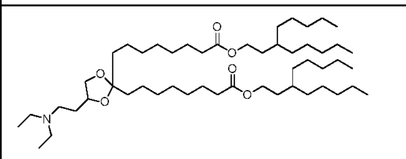
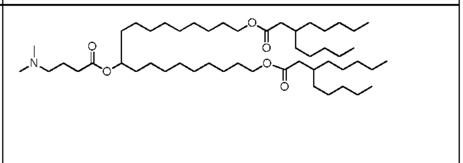
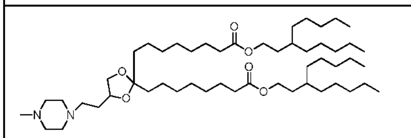
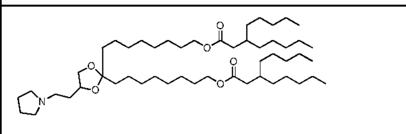
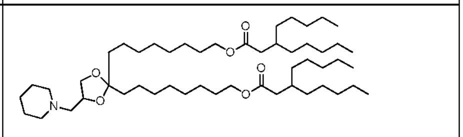
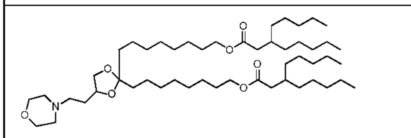
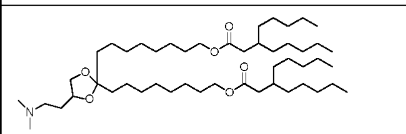
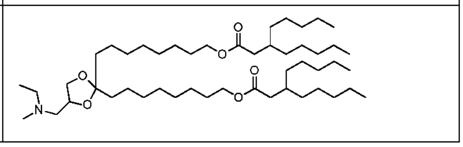
		
		
		
		
		
		
		
		
		
		
		
		
		
		

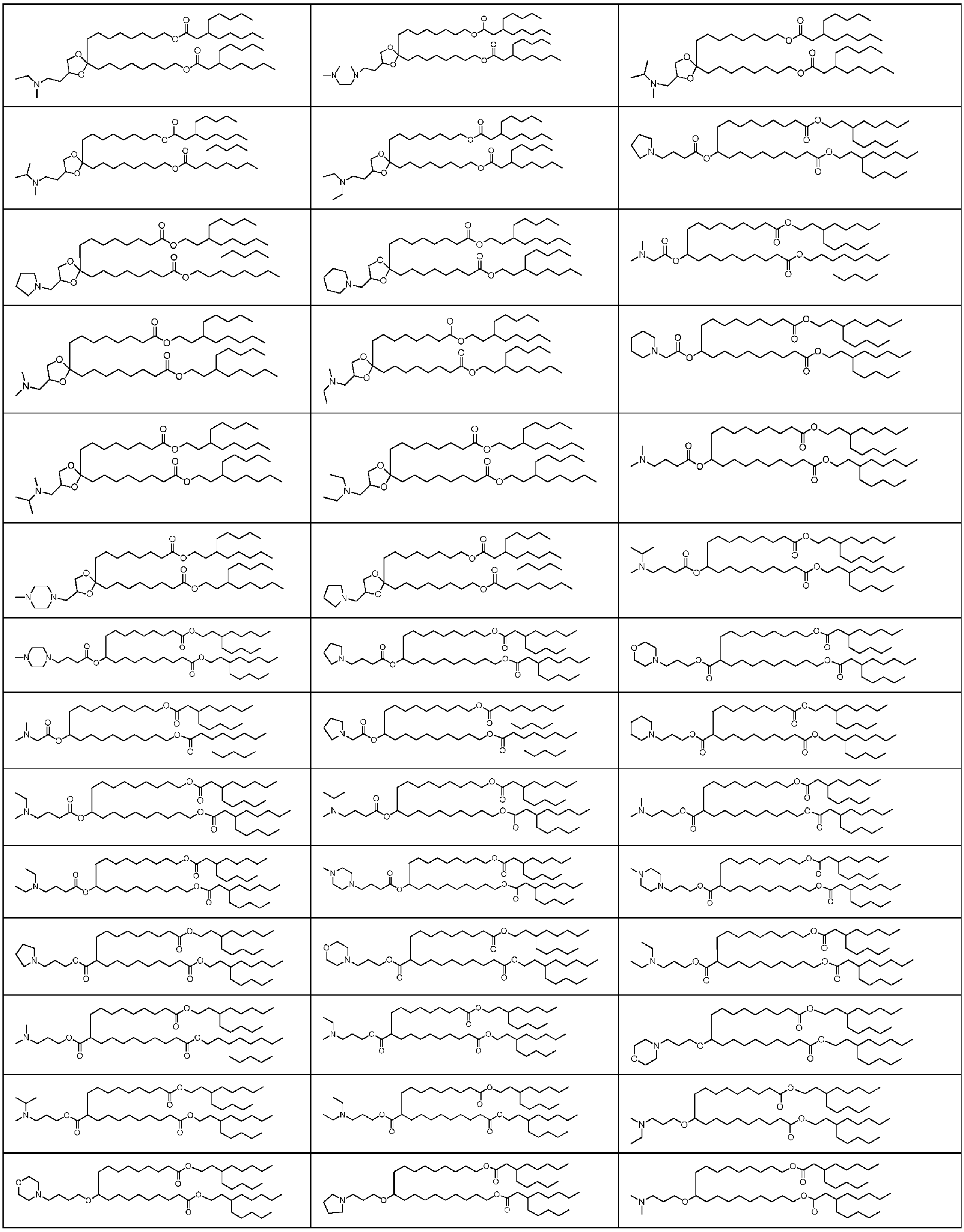


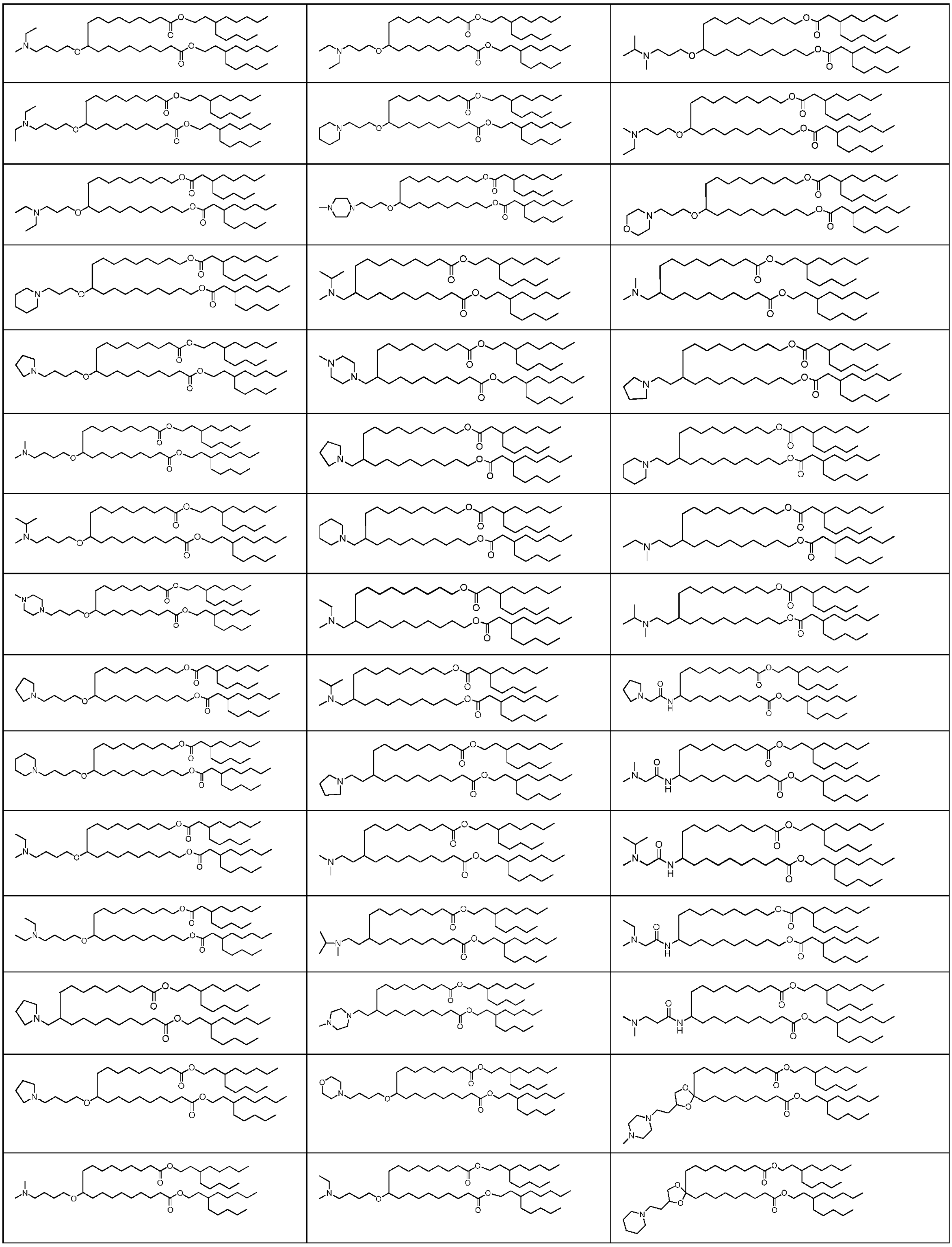
















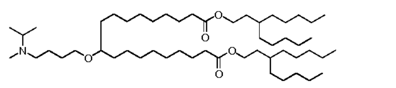
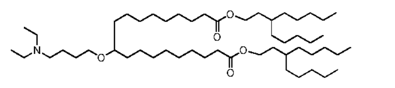
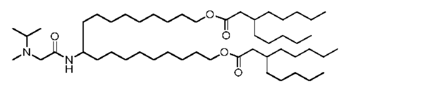
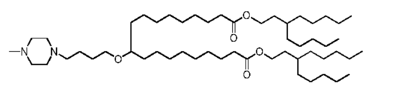
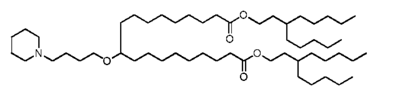
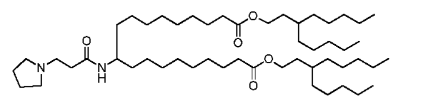
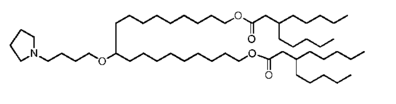
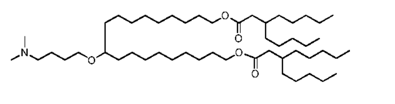
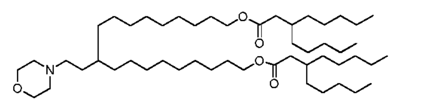
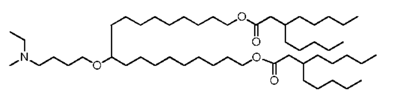
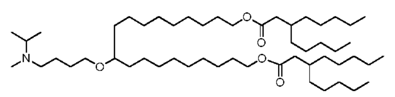
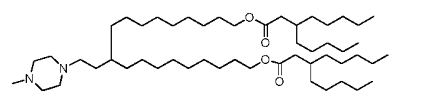
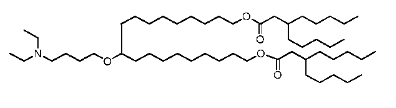
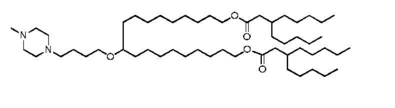
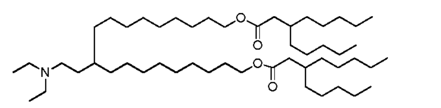
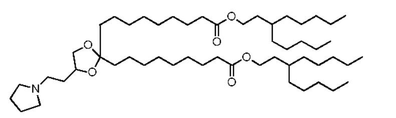
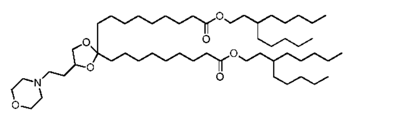
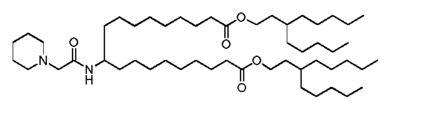
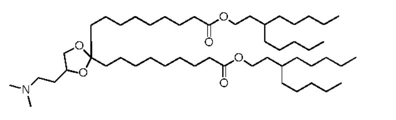
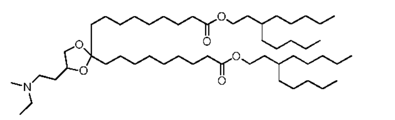
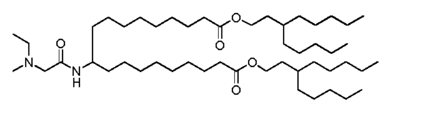
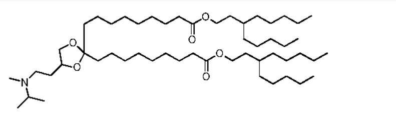
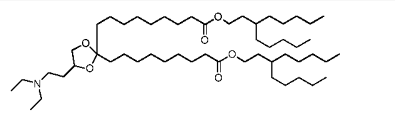
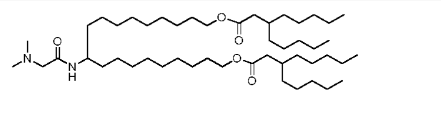
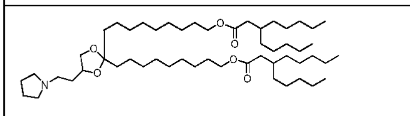
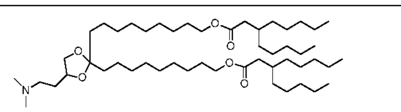
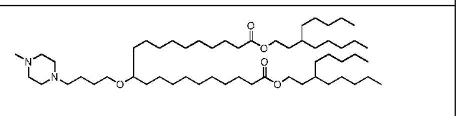
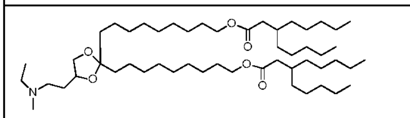
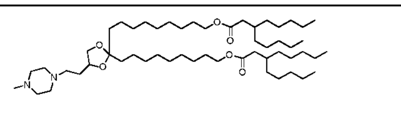
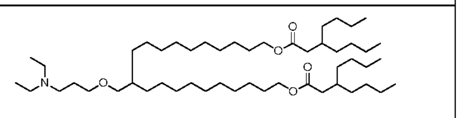
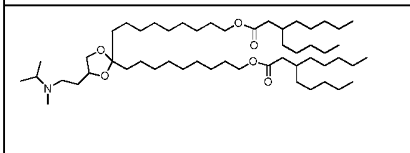
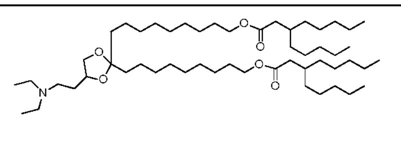
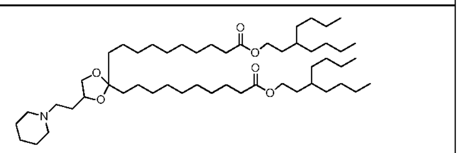
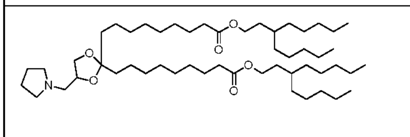
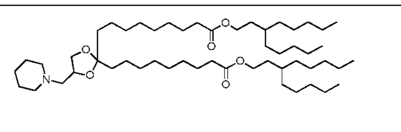
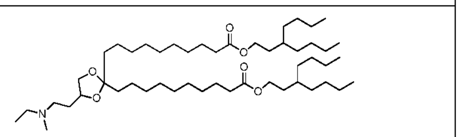
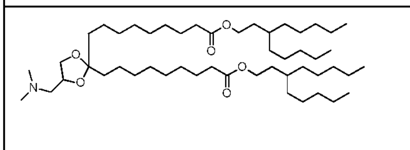
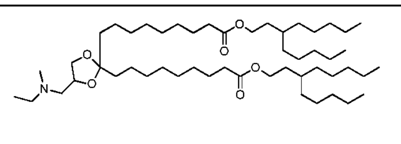
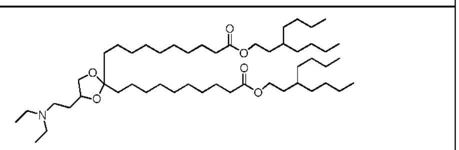
		
		
		
		
		
		
		
		
		
		
		
		
		

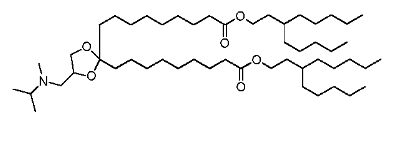
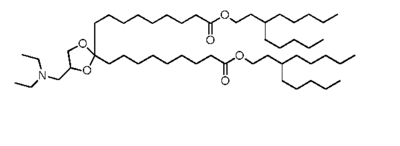
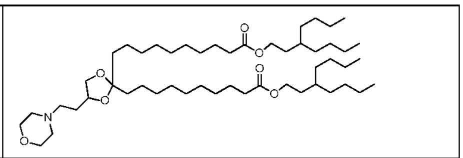
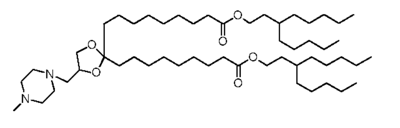
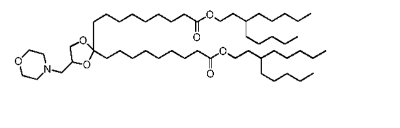
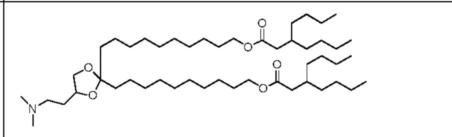
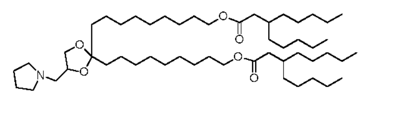
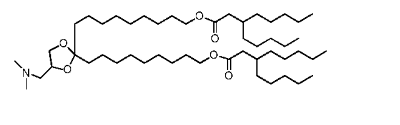
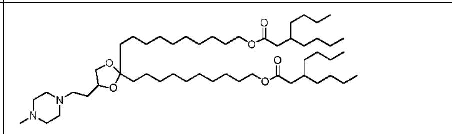
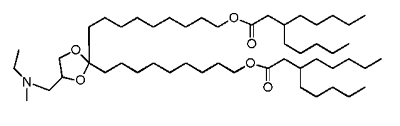
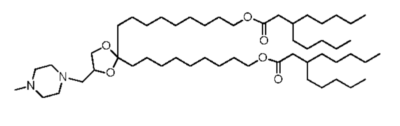
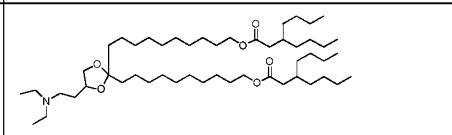
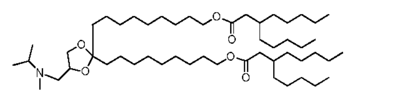
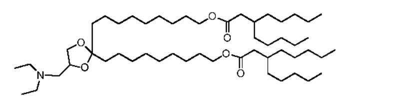
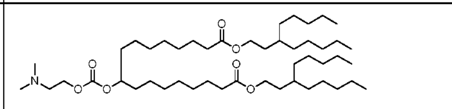
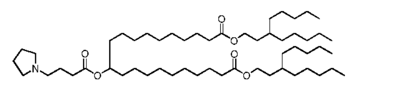
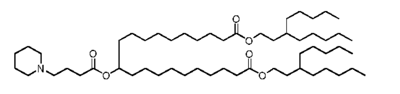
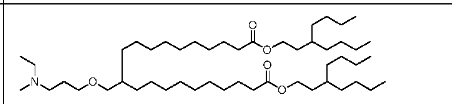
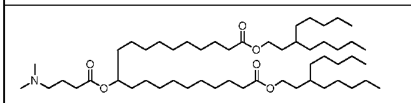
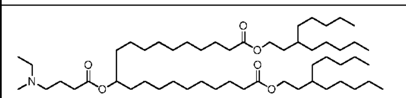
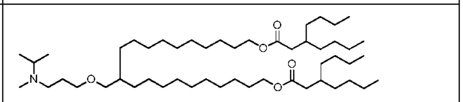
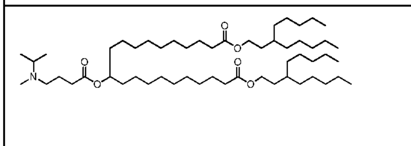
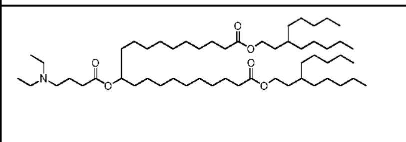
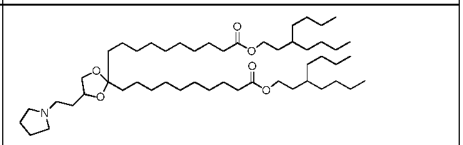
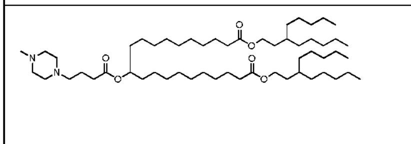
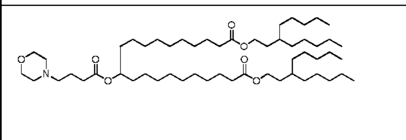
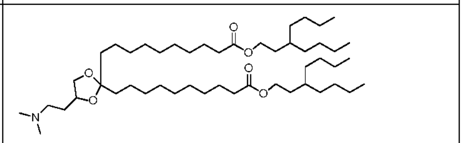
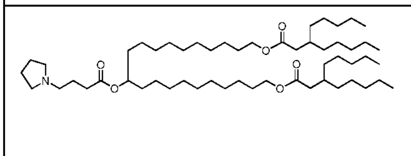
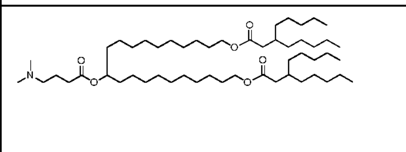
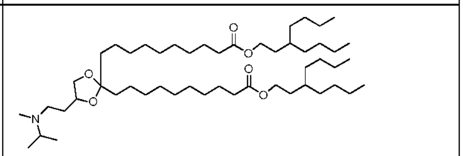
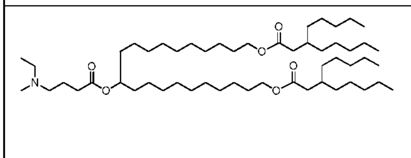
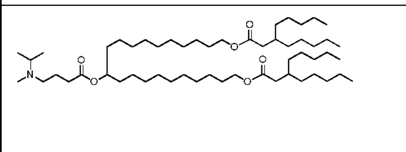
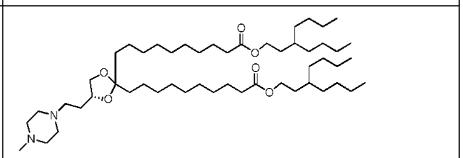
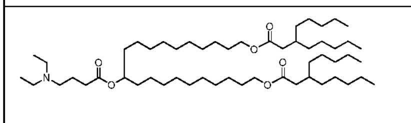
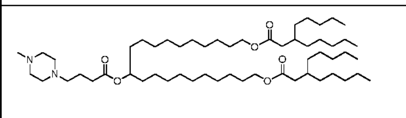
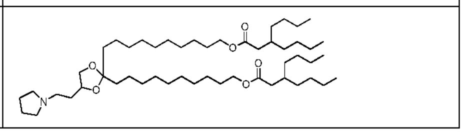


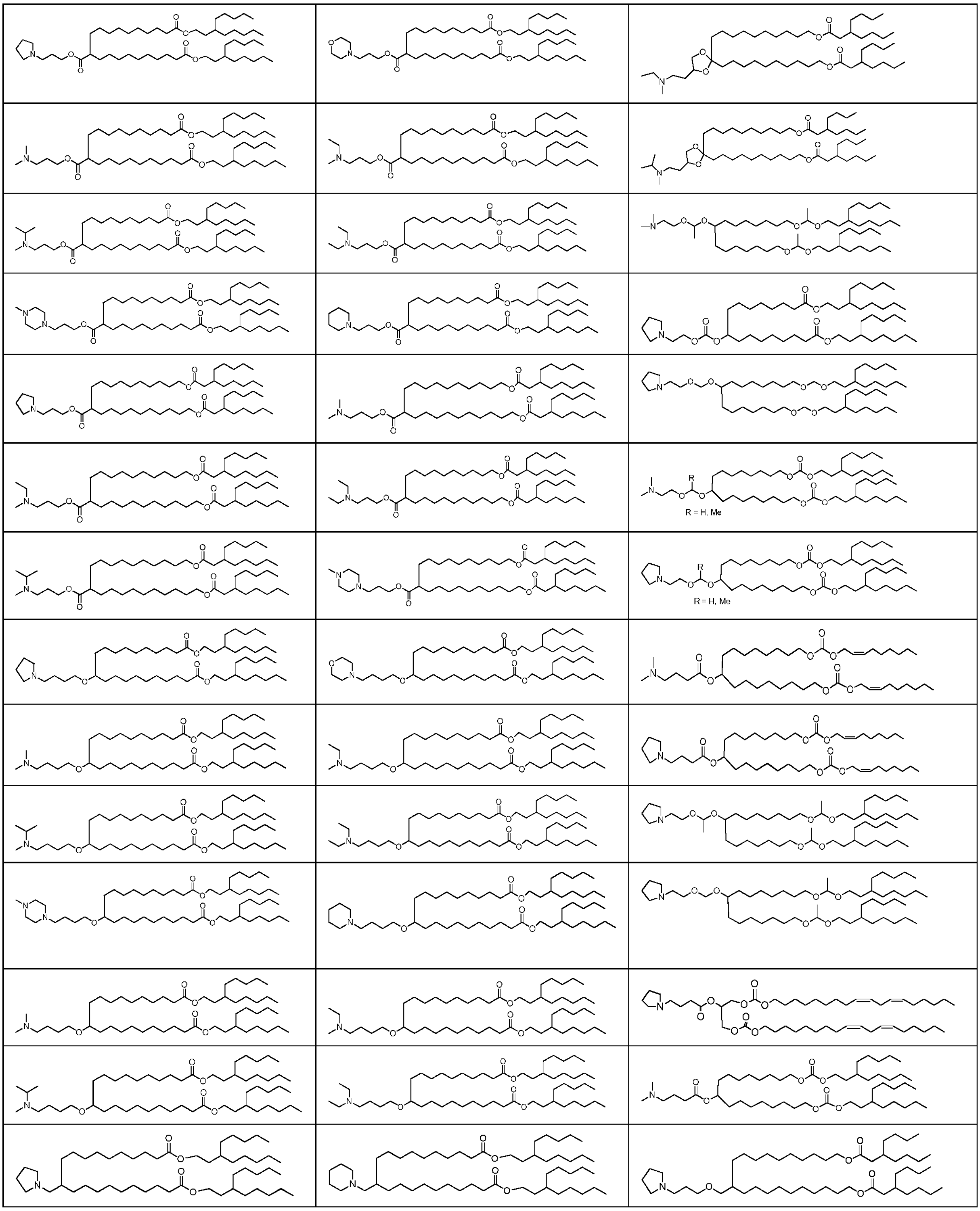
		
		
		
		
		
		
		
		
		
		
		
		

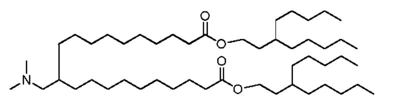
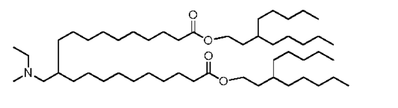
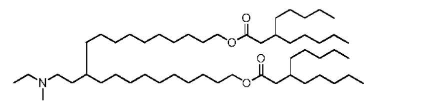
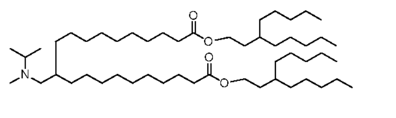
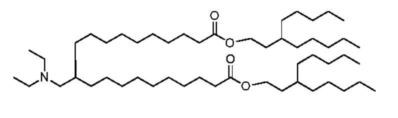
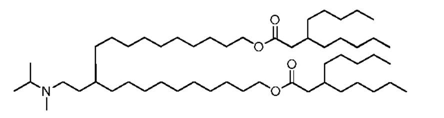
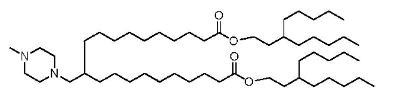
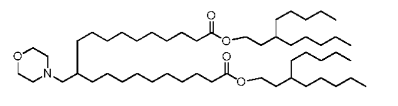
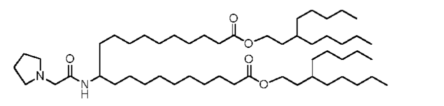
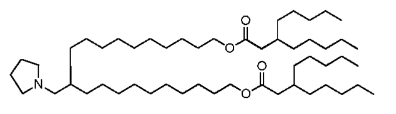
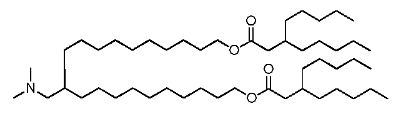
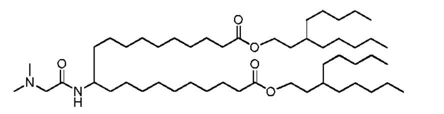
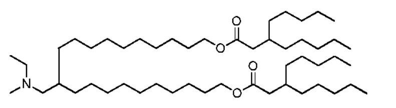
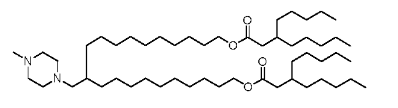
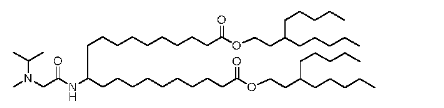
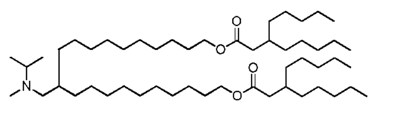
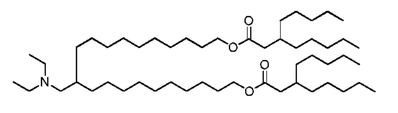
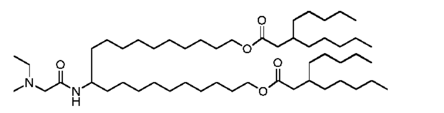
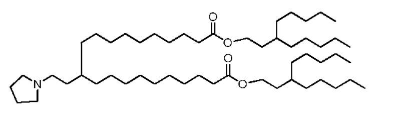
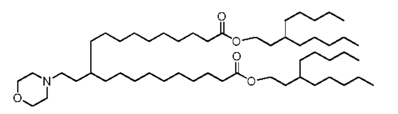
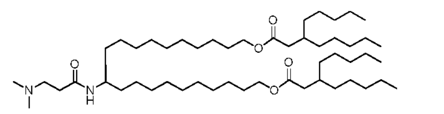
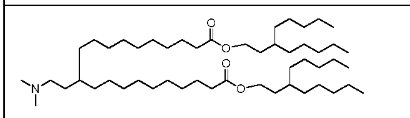
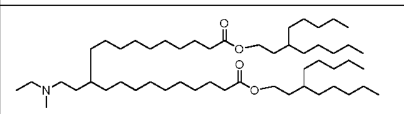
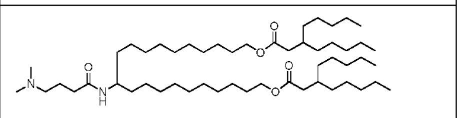
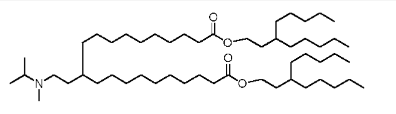
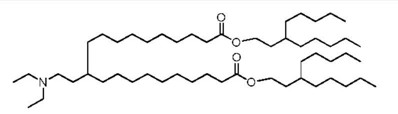
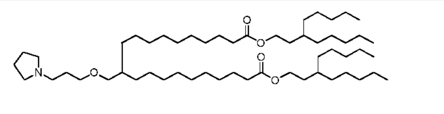
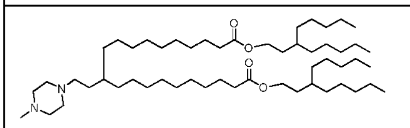
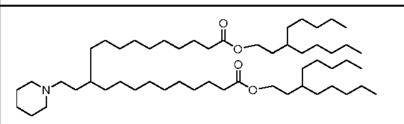
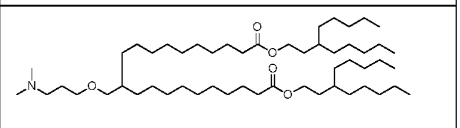
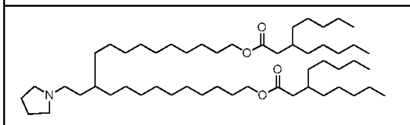
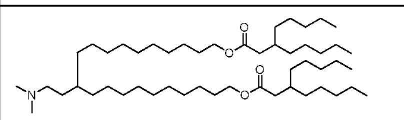
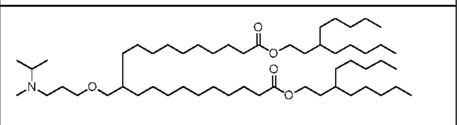
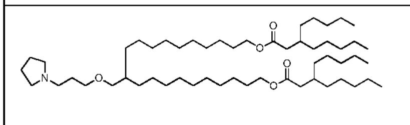
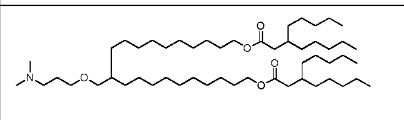
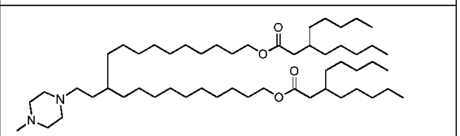
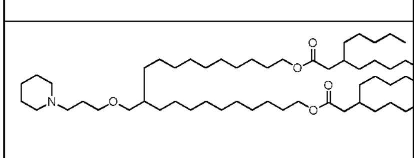
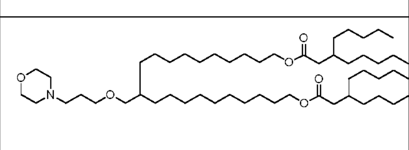
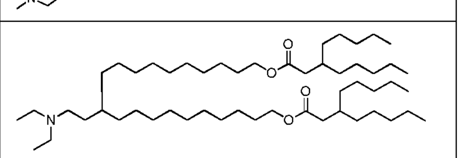


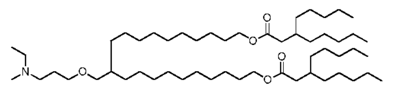
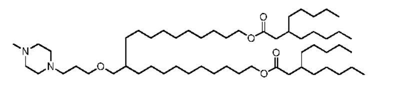
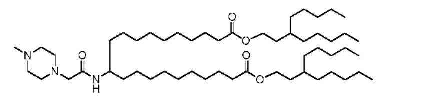
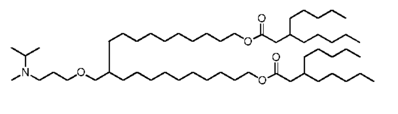
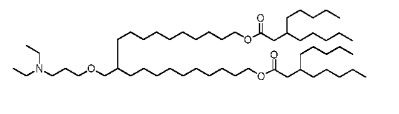
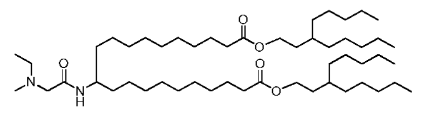
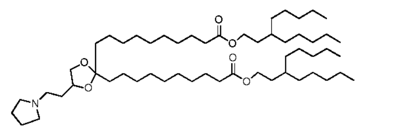
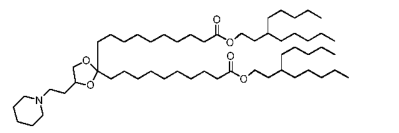
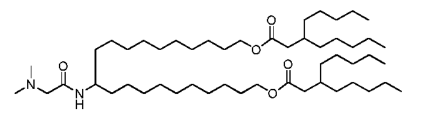
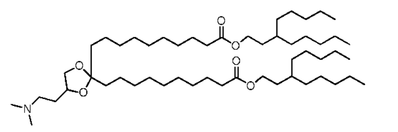
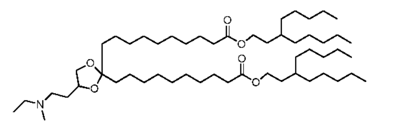
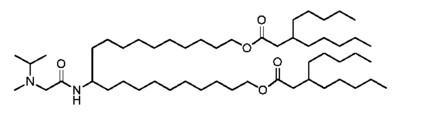
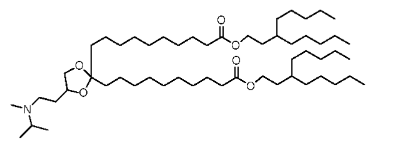
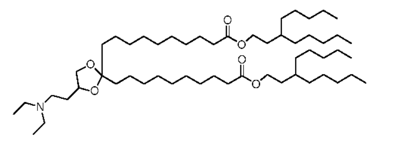
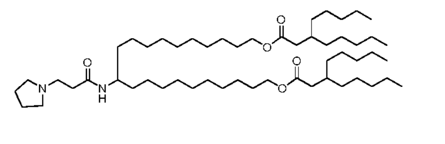
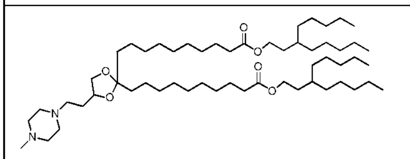
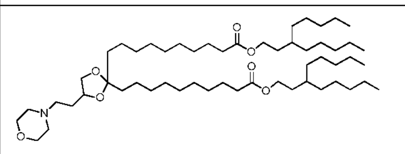
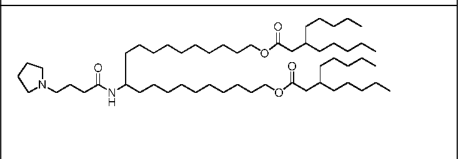
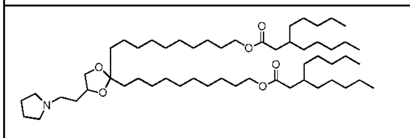
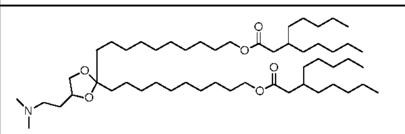
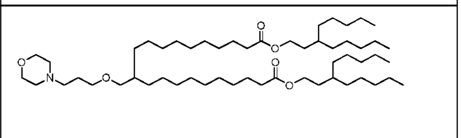
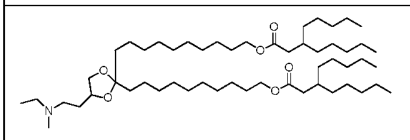
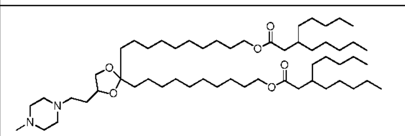
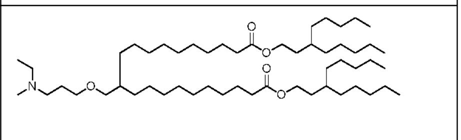
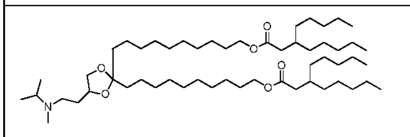
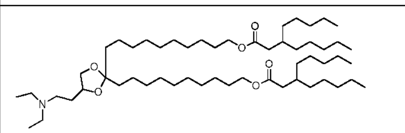
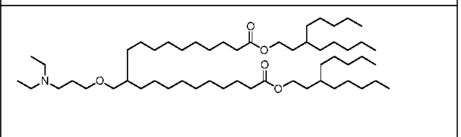
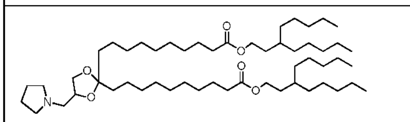
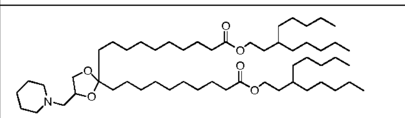
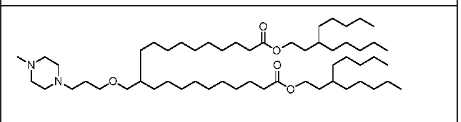
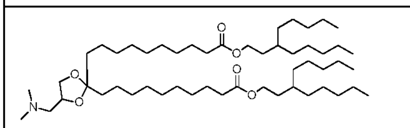
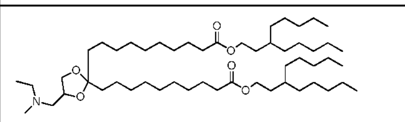
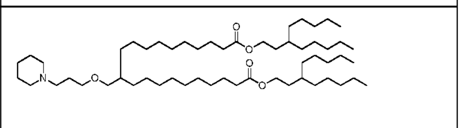
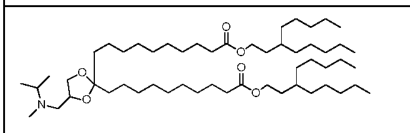
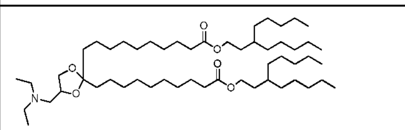
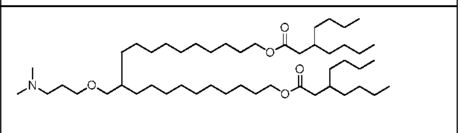


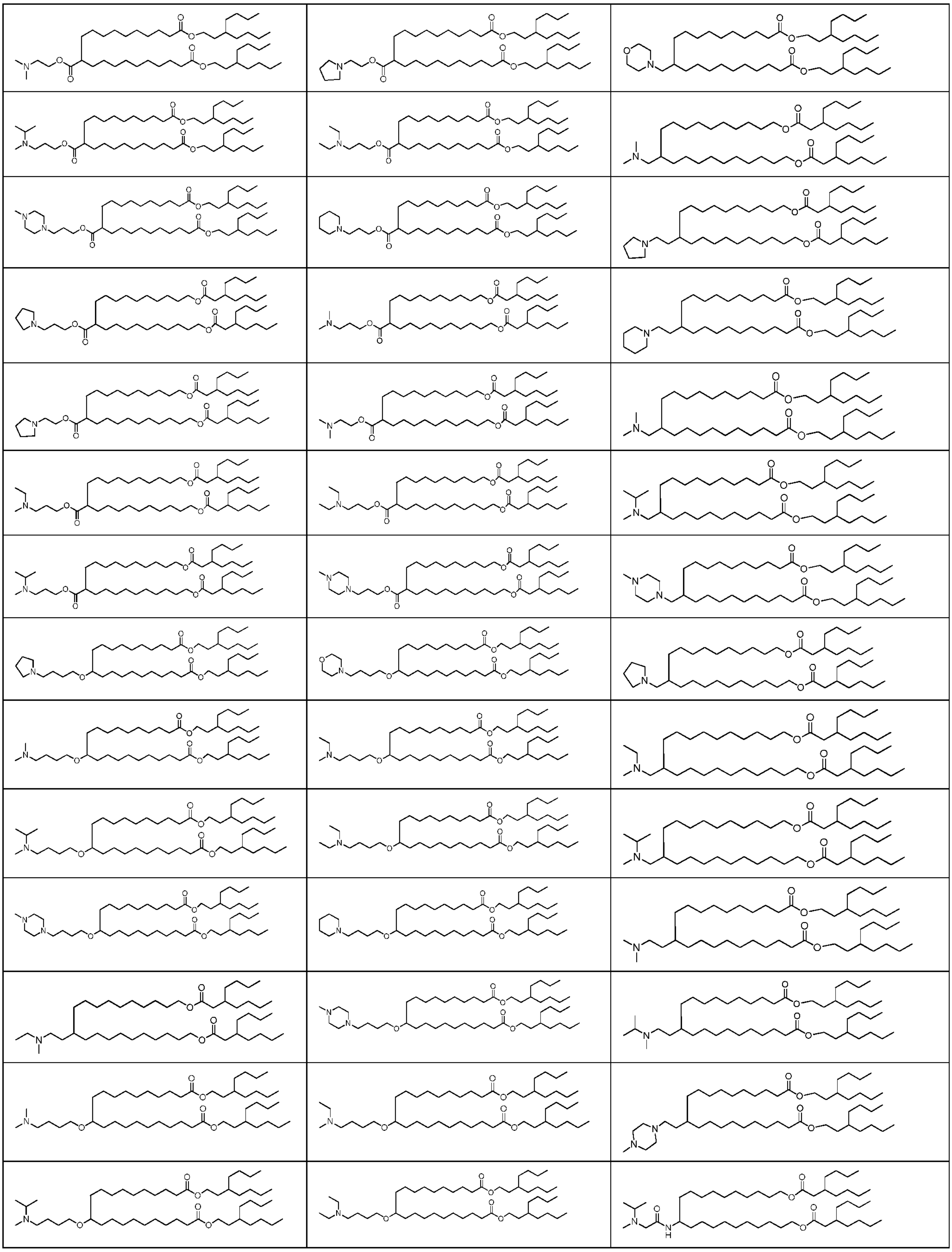
		
		
		
		
		
		
		
		
		
		
		
		
		

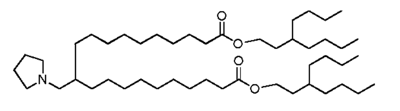
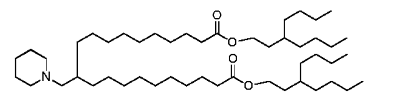
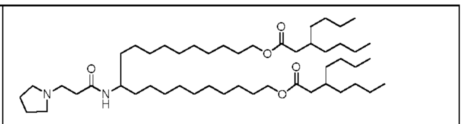
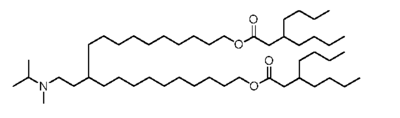
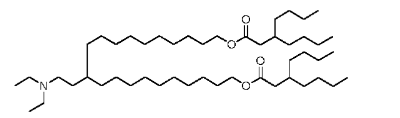
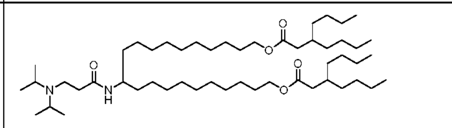
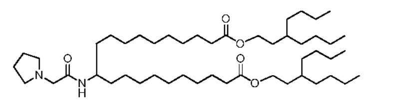
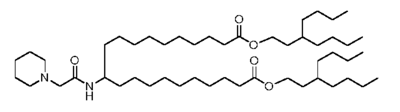
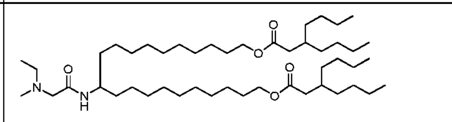
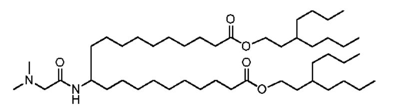
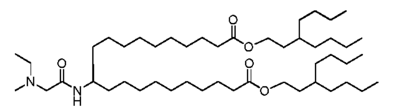
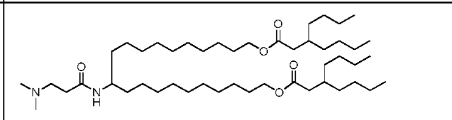
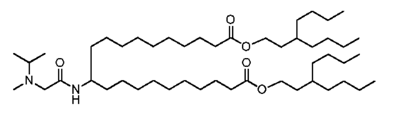
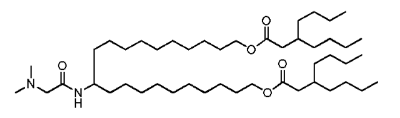
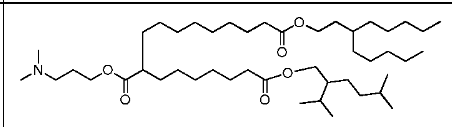
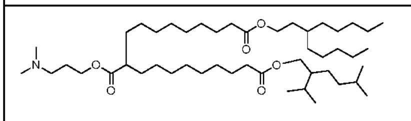
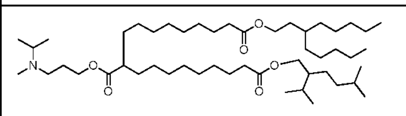
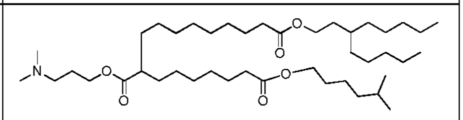
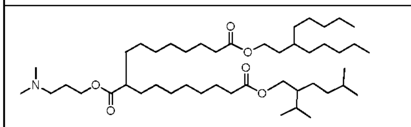
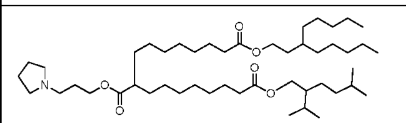
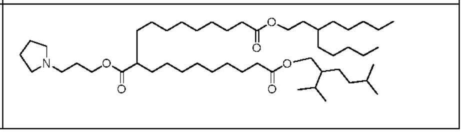
		
		
		
		
		
		
		
		
		
		
		
		


R = H, Me
R = H, Me

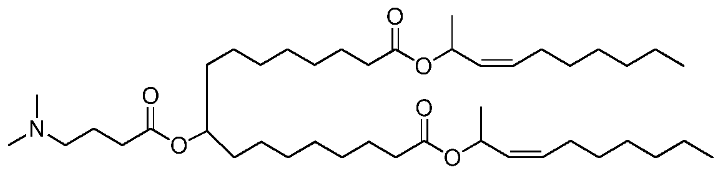
		
		
		
		
		
		
		
		
		
		
		
		

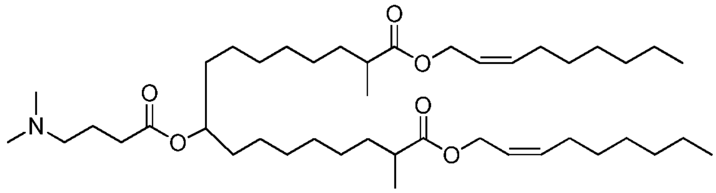


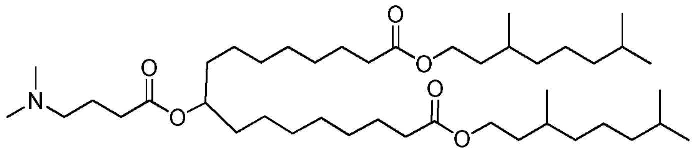
		
		
		
		
		
		
		

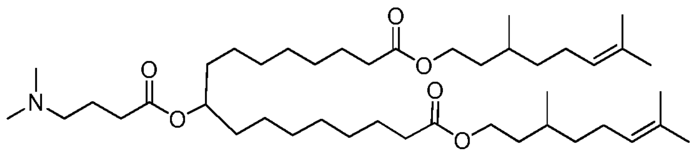
In another aspect, the present invention relates to a method of preparing a compound of Formula I-VII. Suitable exemplary synthetic methods are illustrated in Schemes 1-27 shown in the Examples section below.

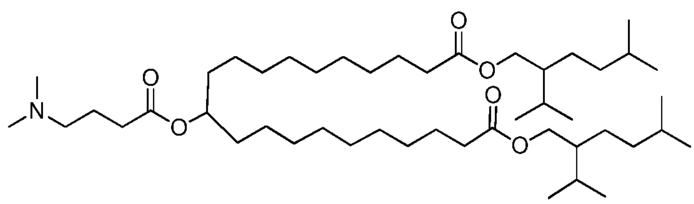
In one embodiment, the cationic lipid of the present invention is selected from the following compounds, and salts thereof (including pharmaceutically acceptable salts thereof). These cationic lipids are suitable for forming nucleic acid-lipid particles.

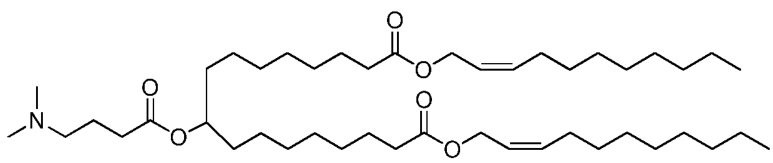


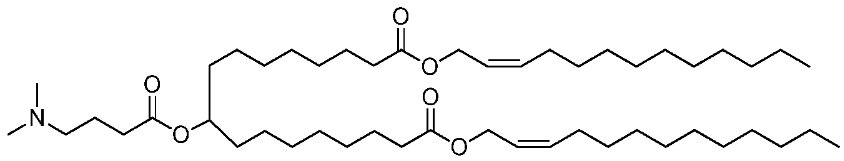


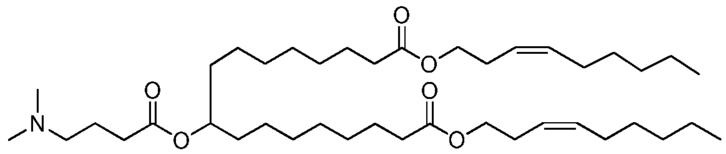


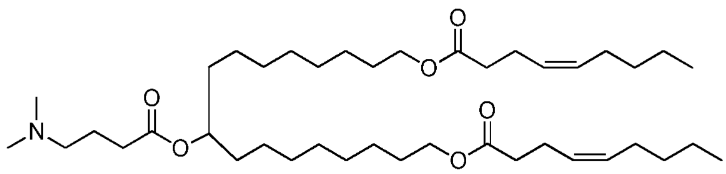


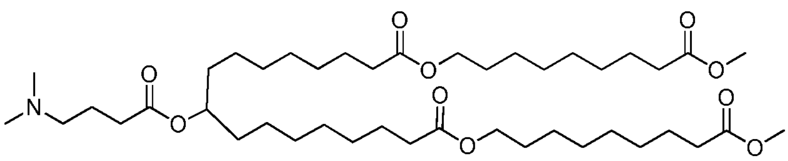


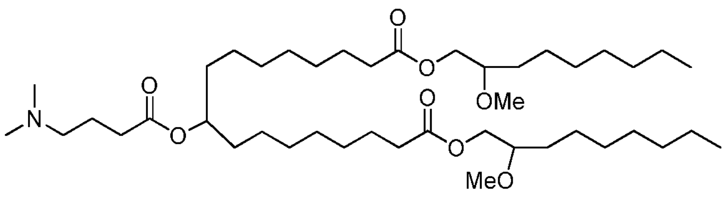


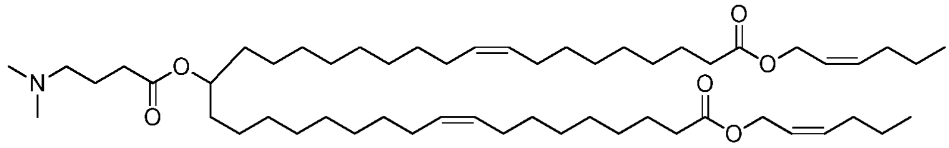


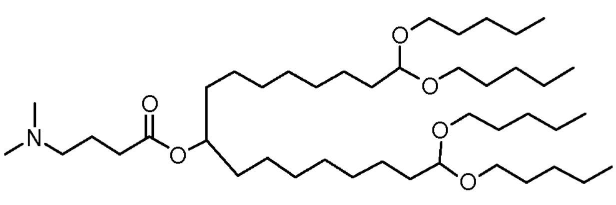


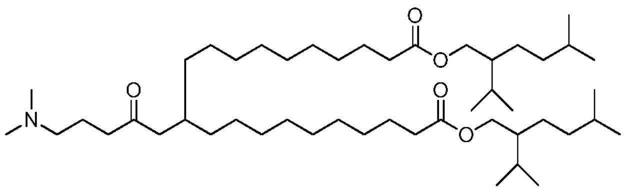


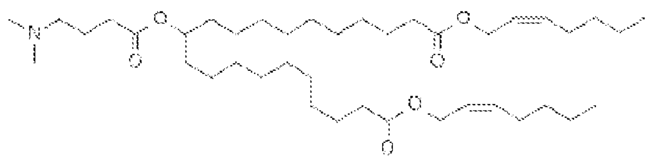


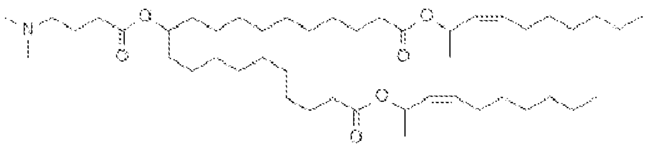


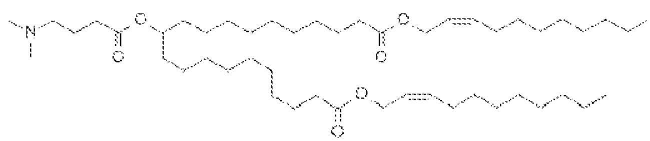


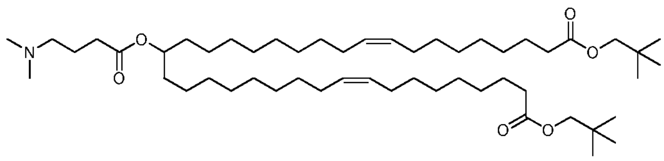


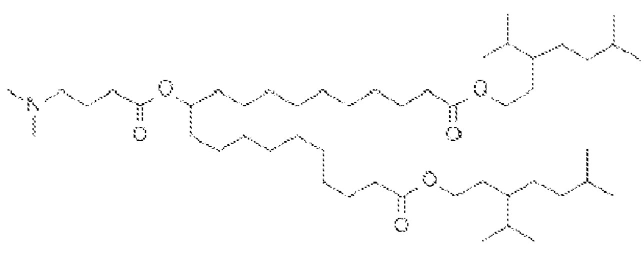


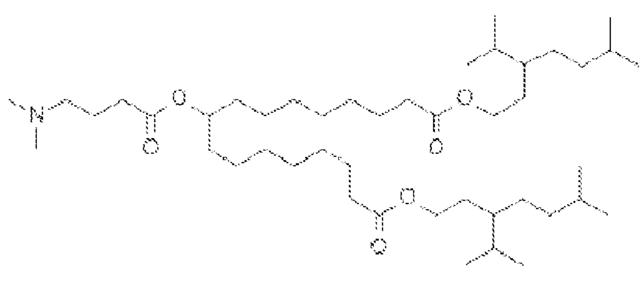


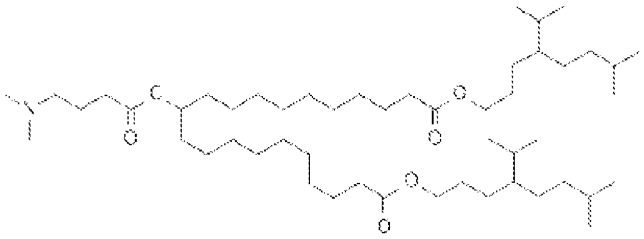


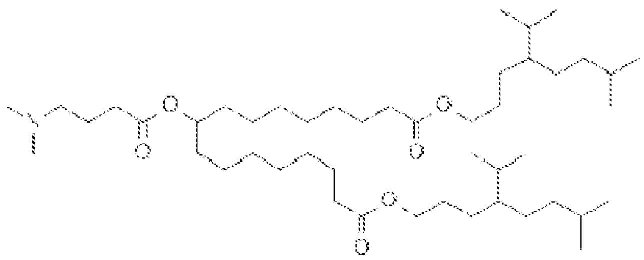


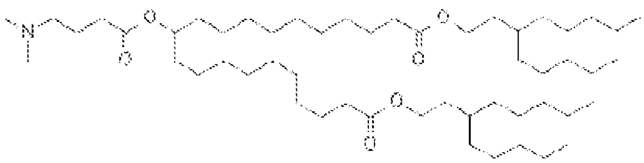


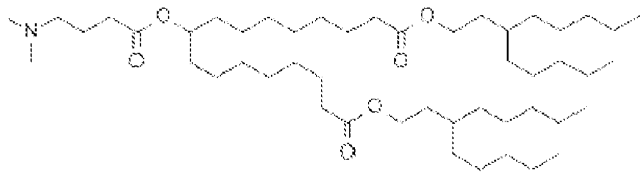




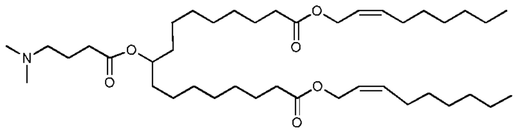


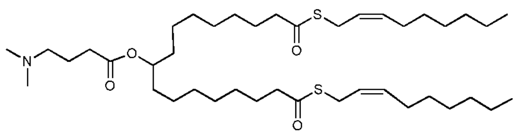


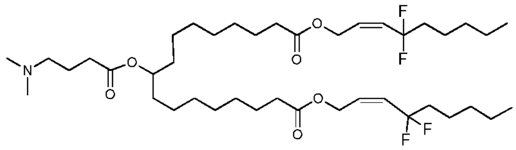


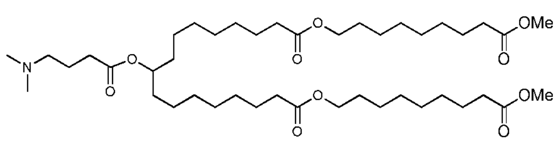


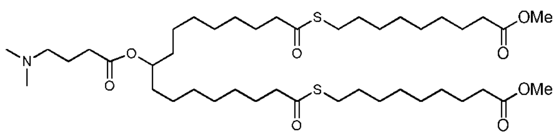
In another embodiment, the cationic lipid of the present invention is selected from the following compounds, and salts thereof (including pharmaceutically acceptable salts thereof):

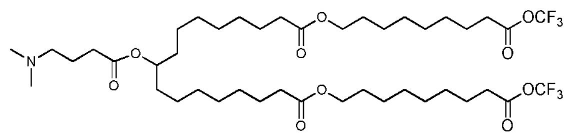


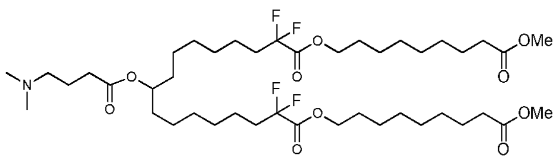


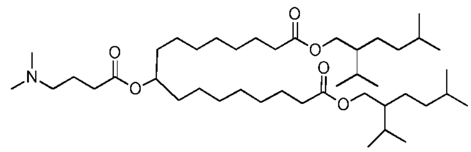


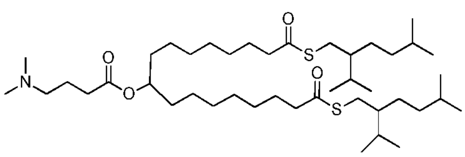


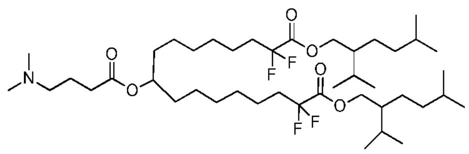


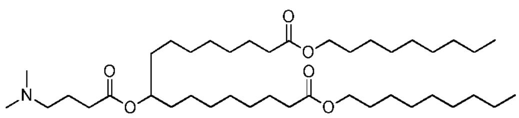


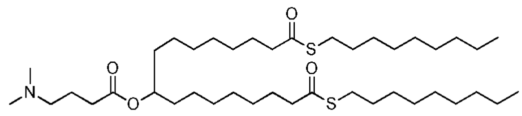


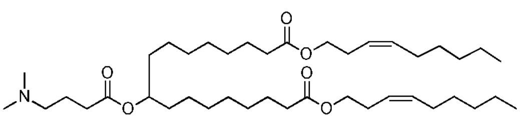


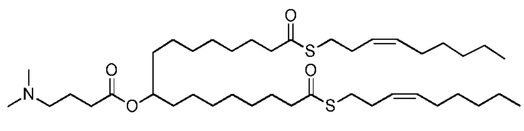


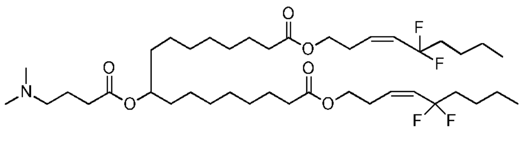


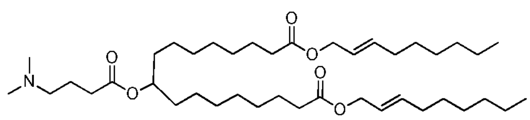


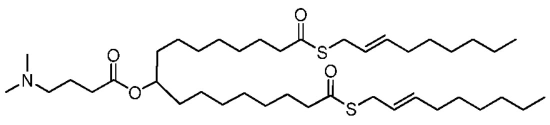
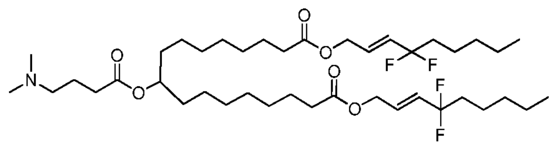
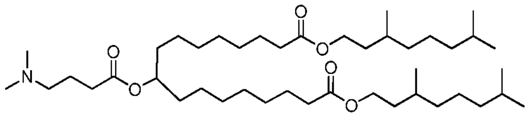
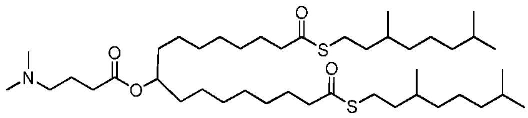
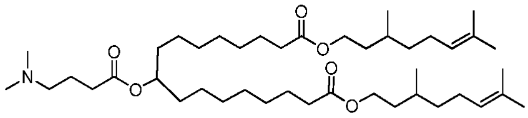
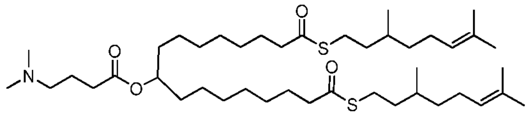
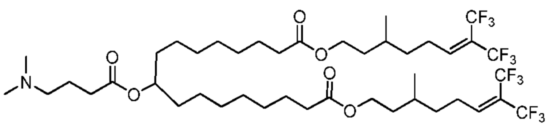
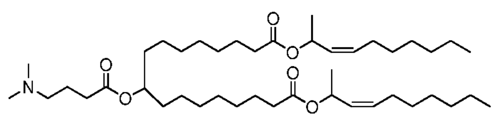
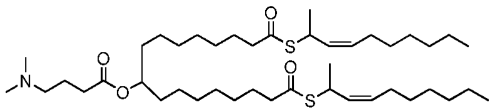
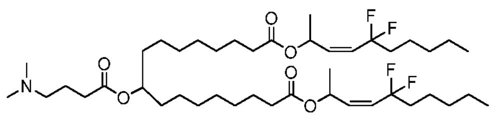
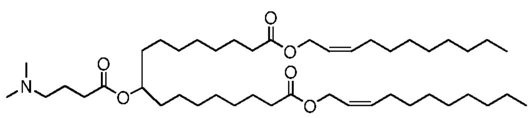
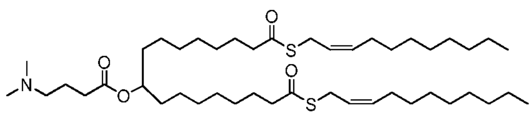
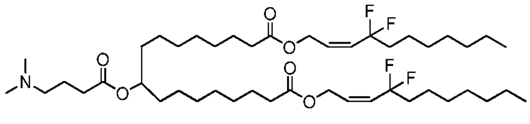
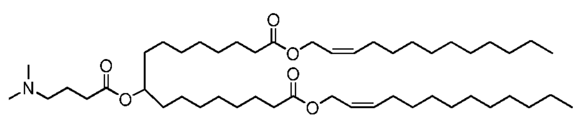
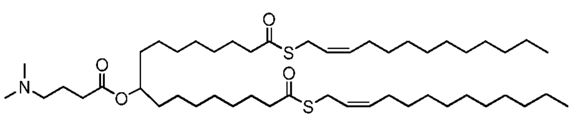
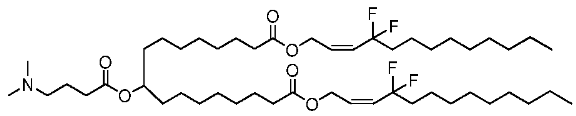


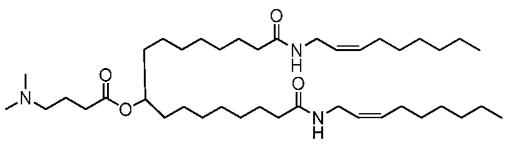
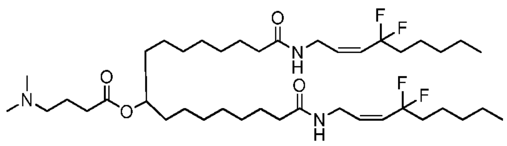
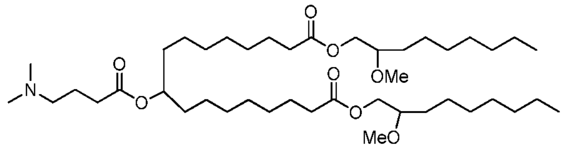
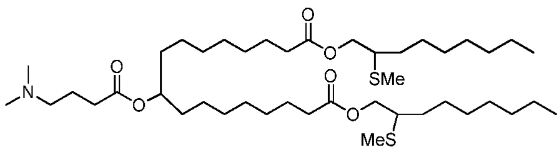
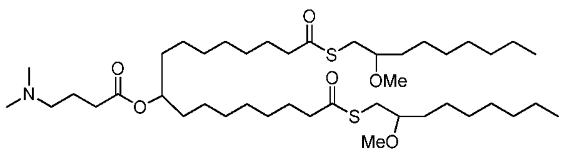
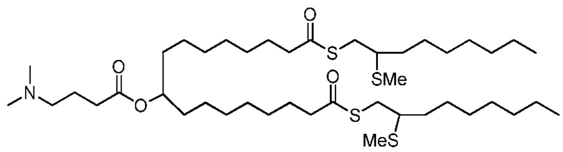
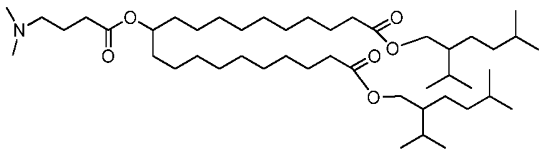
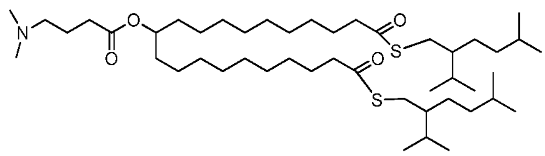
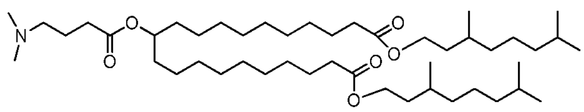
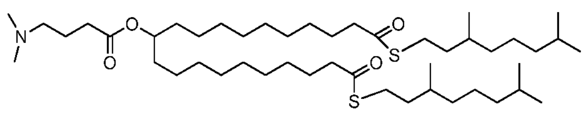
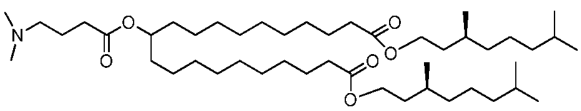
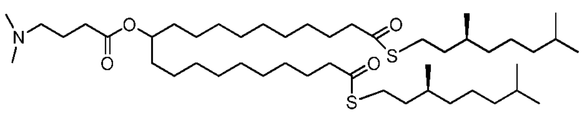




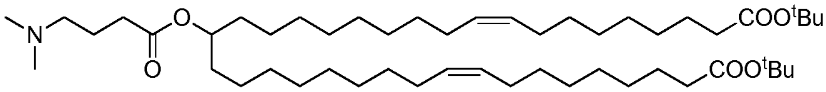


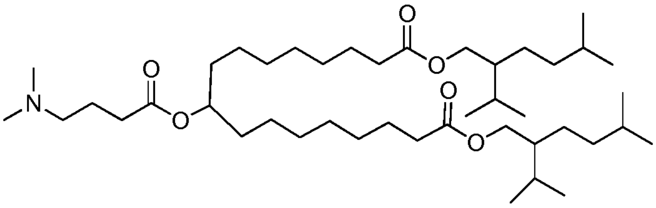


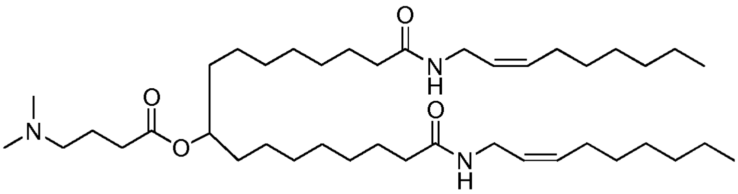

















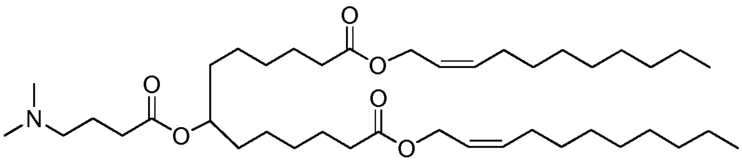













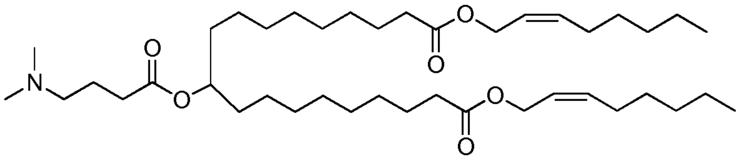
In another embodiment, the cationic lipid of the present invention is selected from the following compounds, and salts thereof (including pharmaceutically acceptable salts thereof):

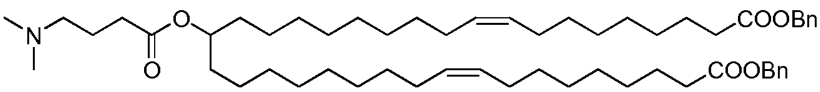

COO^{t}Bu
COO^{t}Bu

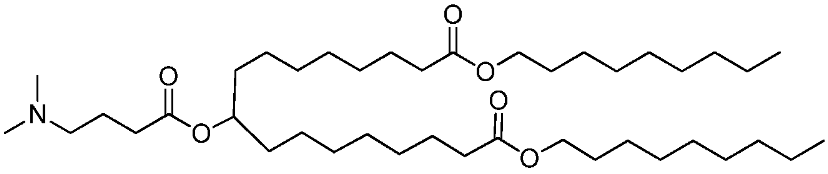


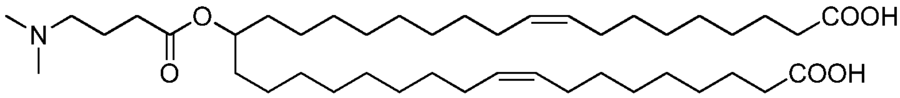


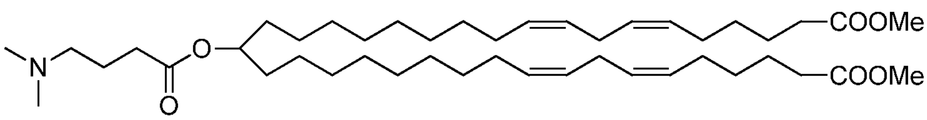


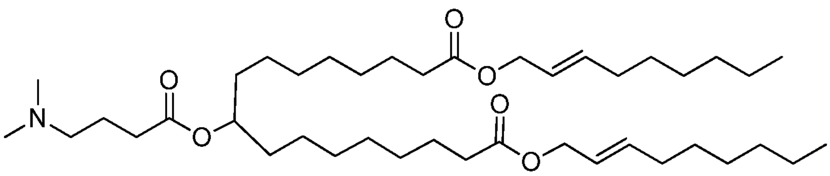



COOBn
COOBn

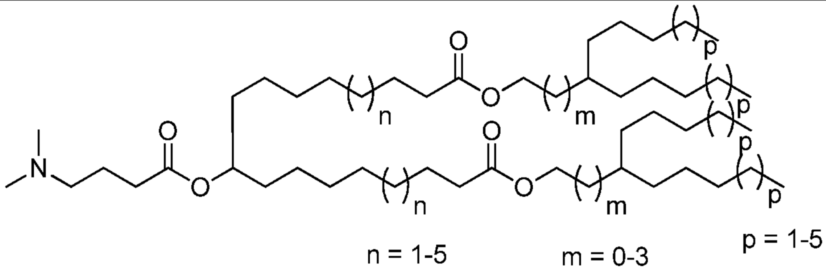
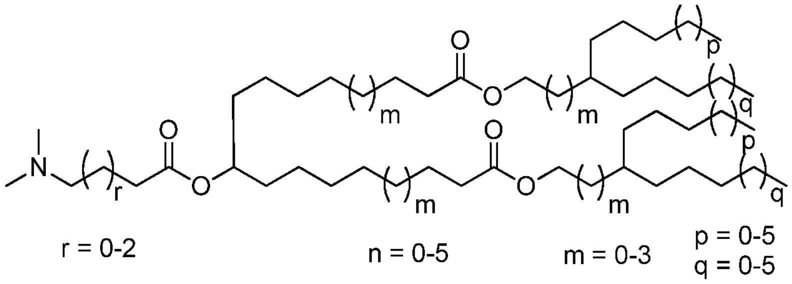


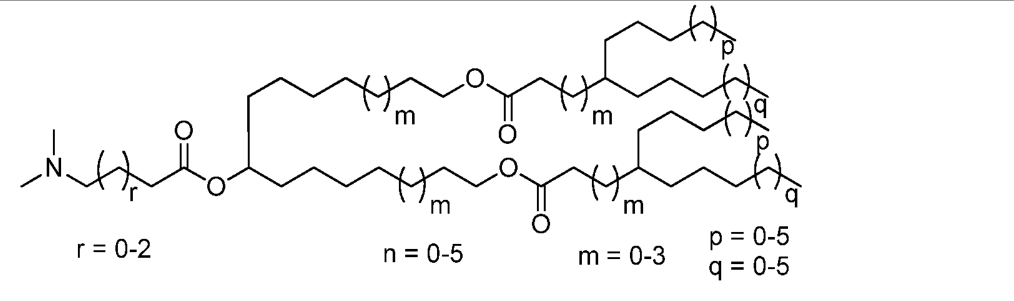


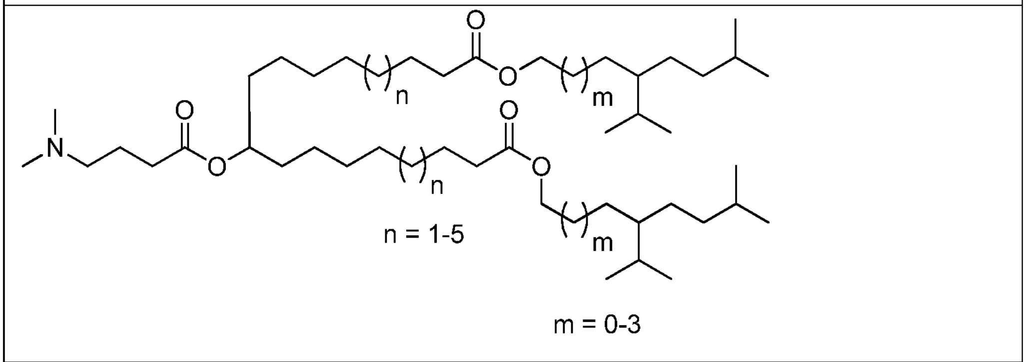


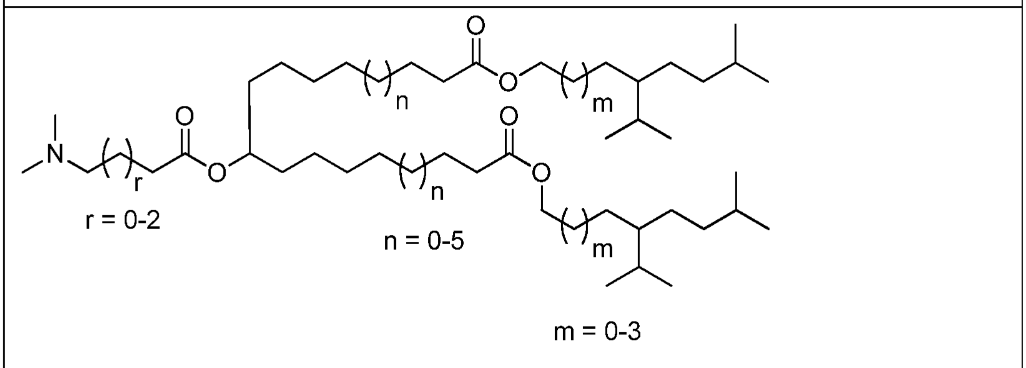


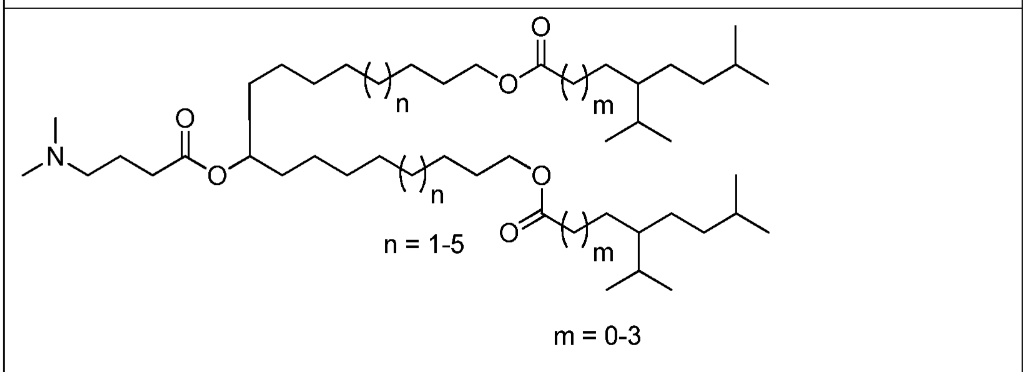
Additional representative cationic lipids include, but are not limited to:

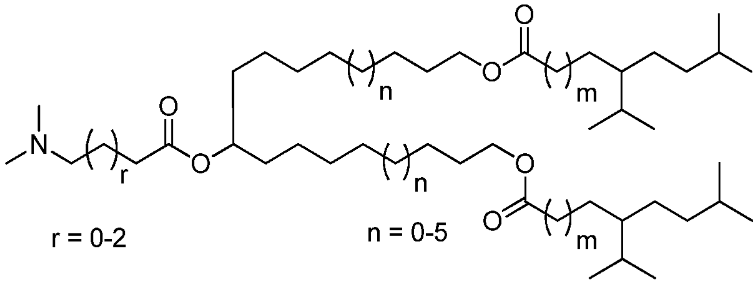
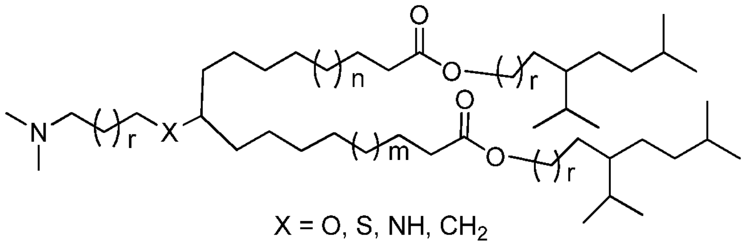
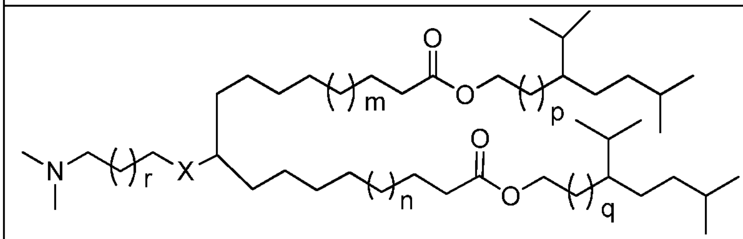
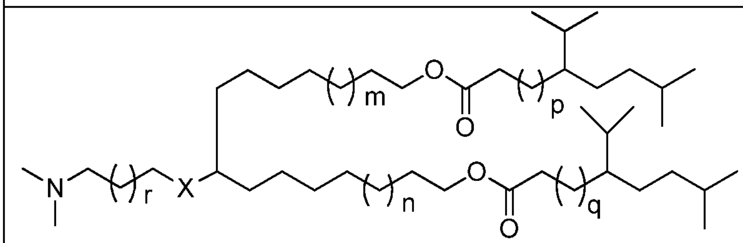



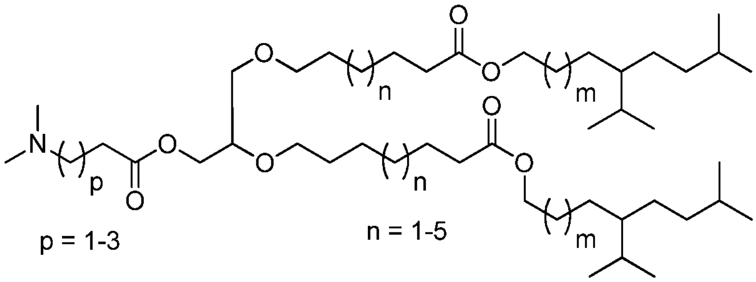

O
O
O
N
O
O
O
m
m
m
m
r
p
q
p
q
O
r = 0-2
n = 0-5
m = 0-3
p = 0-5
q = 0-5


N
O
O
O
O
O
O
n
n
m
m
n = 1-5
m = 0-3

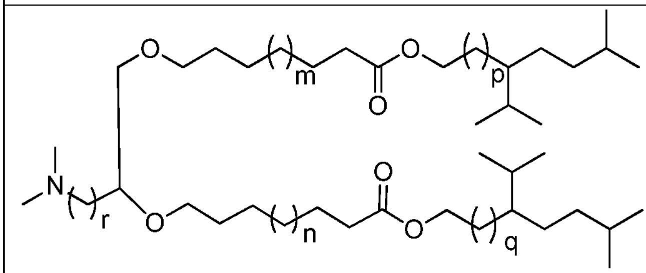

N
O
O
O
O
O
O
r
n
n
m
m
r = 0-2
n = 0-5
m = 0-3


N
O
O
O
O
O
O
n
n
m
m
n = 1-5
m = 0-3

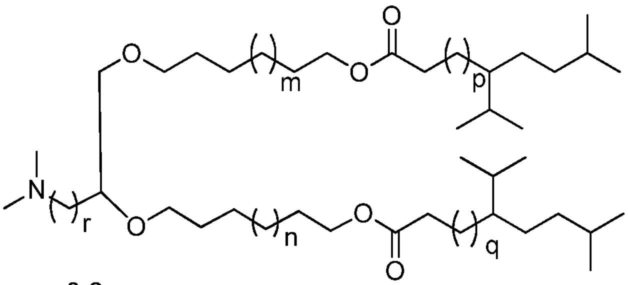
 r = 0-2 n = 0-5 m = 0-3
 X = O, S, NH, CH_2 r = 0-2, n = 1-5, and m = 1-5
 r = 0-2 X = O, S, NH, CH_2 m = 0-5 p = 0-3 n = 0-5 q = 0-3
 r = 0-2 X = O, S, NH, CH_2 m = 0-5 p = 0-3 n = 0-5 q = 0-3



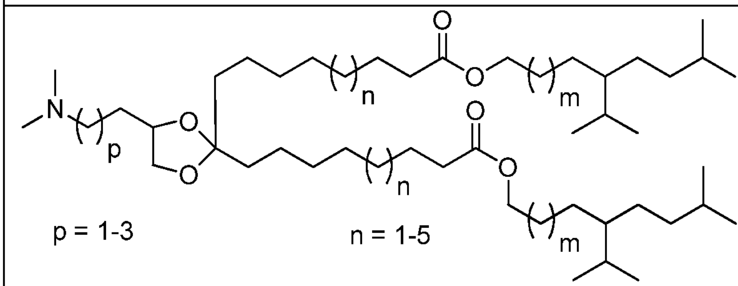
p = 1-3 n = 1-5 m = 0-3



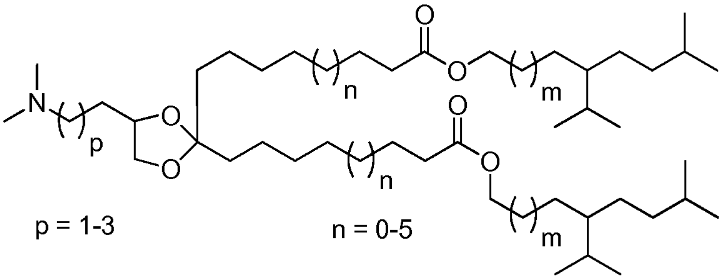
r = 0-2 m = 0-5 n = 0-5 p = 0-3 q = 0-3



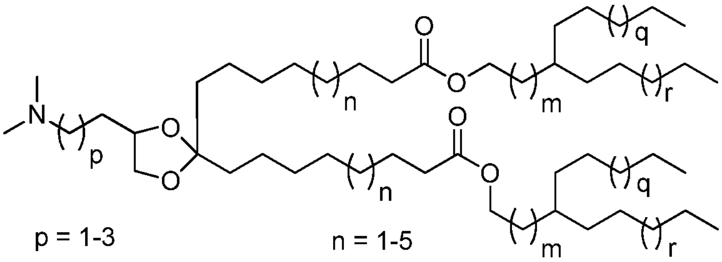
r = 0-2 m = 0-5 n = 0-5 p = 0-3 q = 0-3



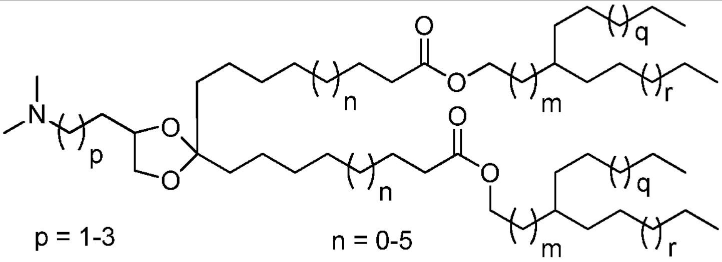
p = 1-3 n = 1-5 m = 0-3



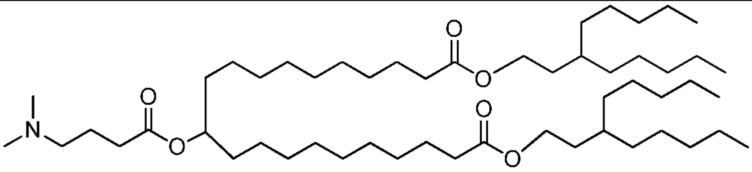
p = 1-3 n = 0-5 m = 0-3

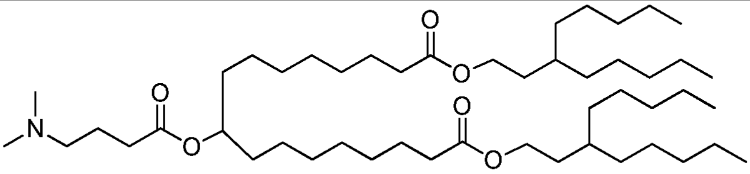


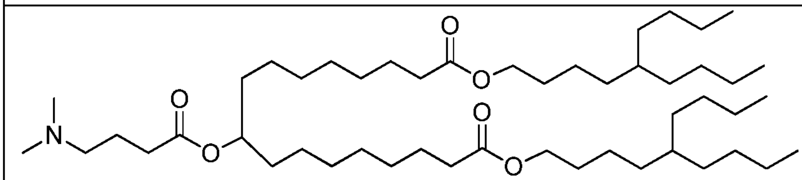
p = 1-3 n = 1-5 m = 0-3; q = 0-4; r = 0-4

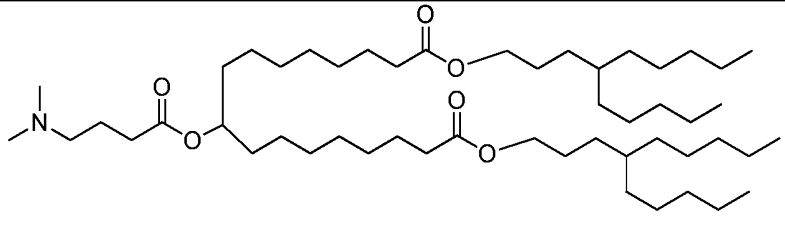
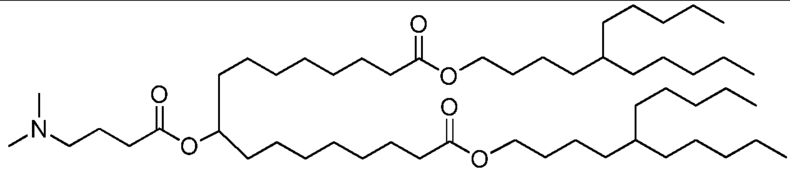
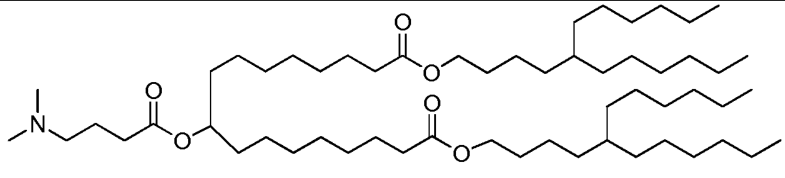
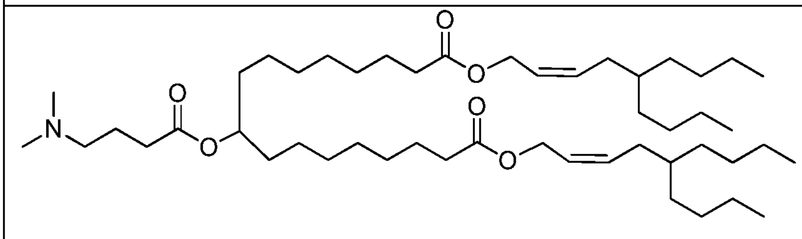
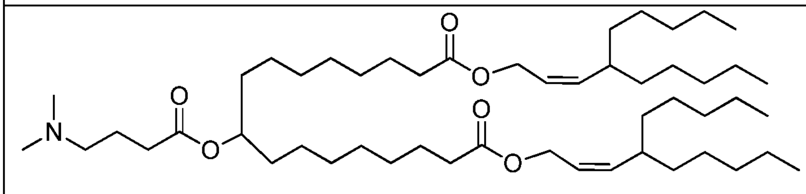
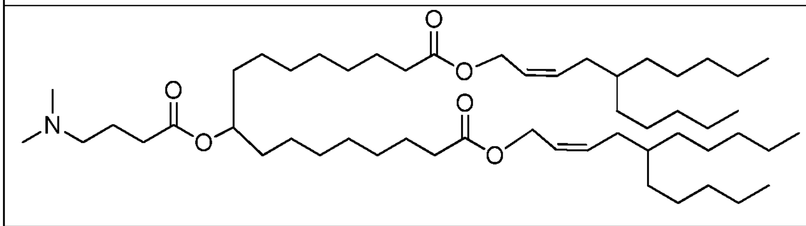
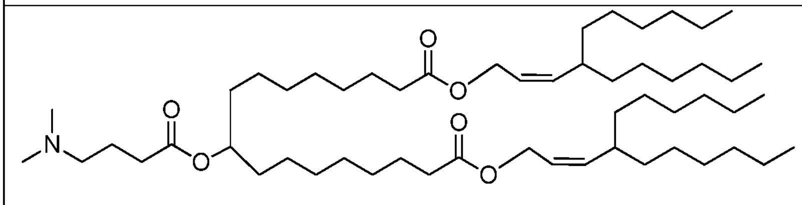
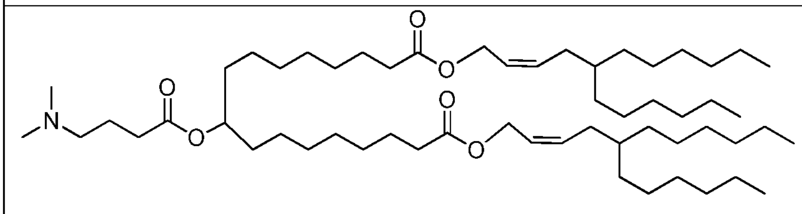


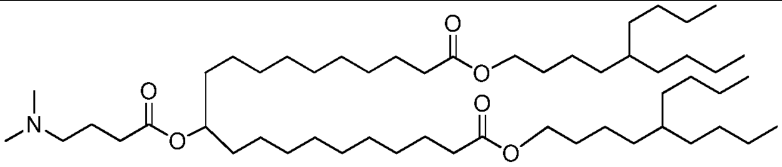
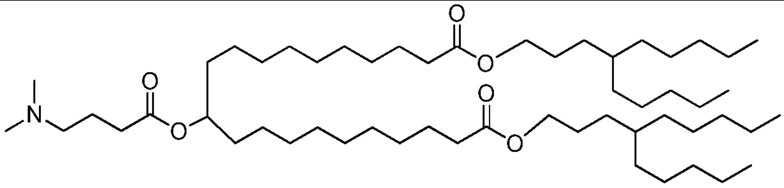
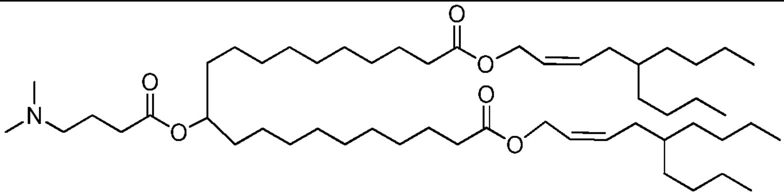
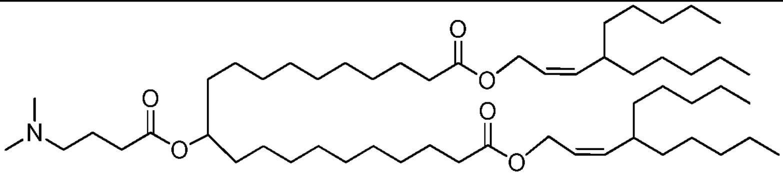
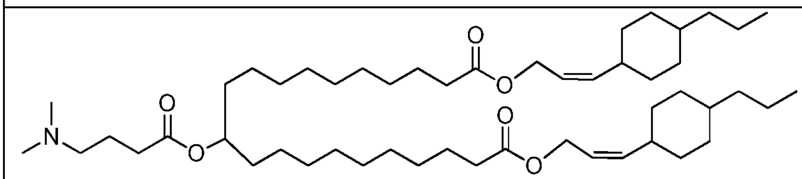
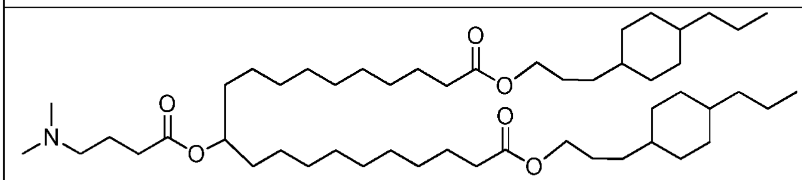
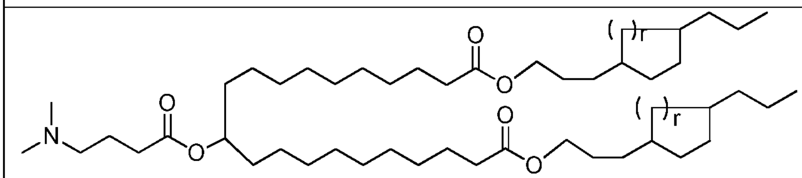
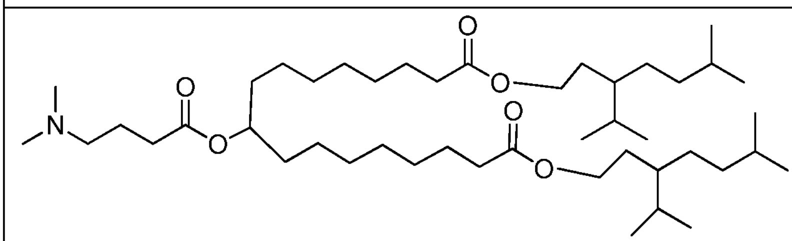
p = 1-3 n = 0-5 m = 0-3; q = 0-4; r = 0-4

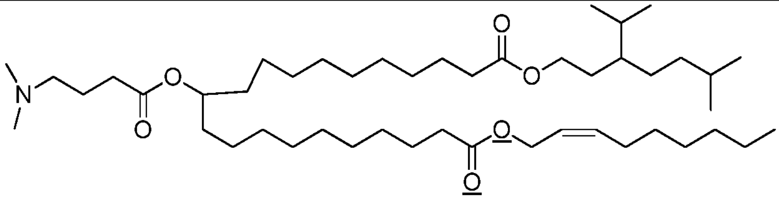
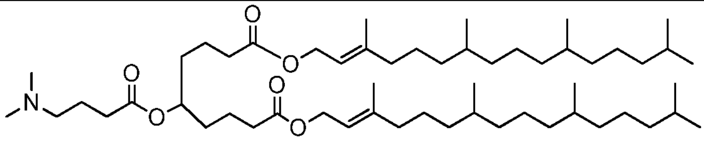
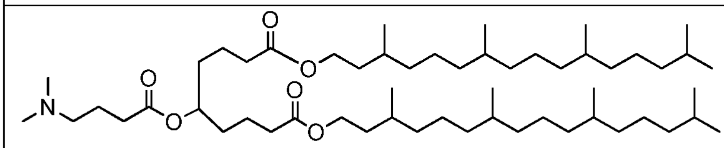
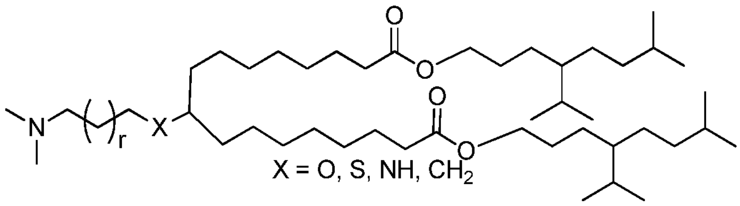
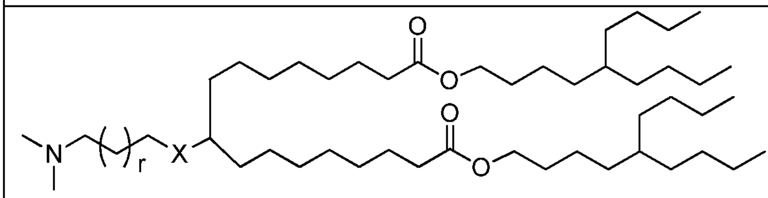


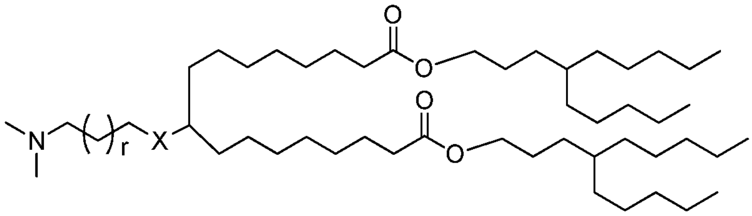
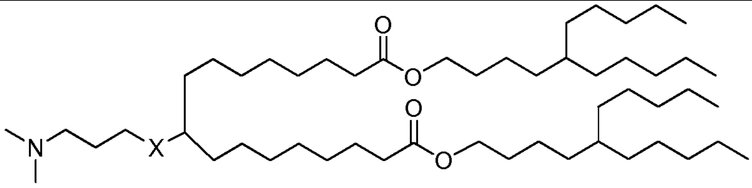
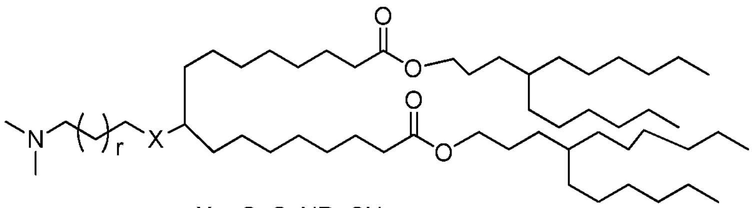
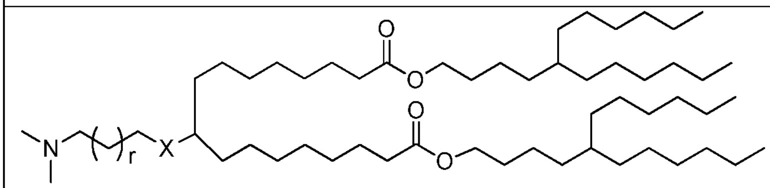
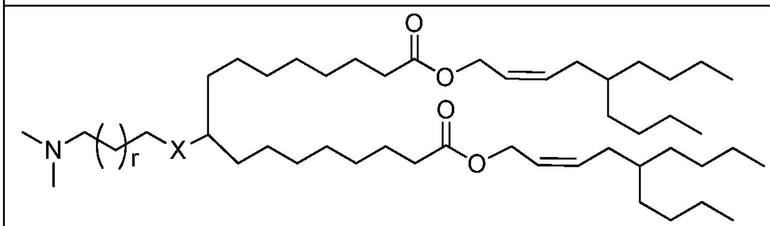


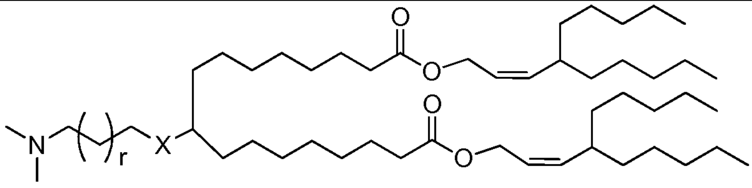
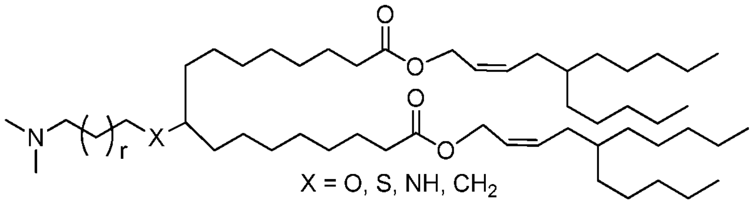
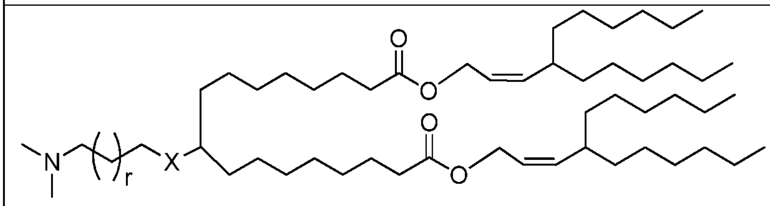
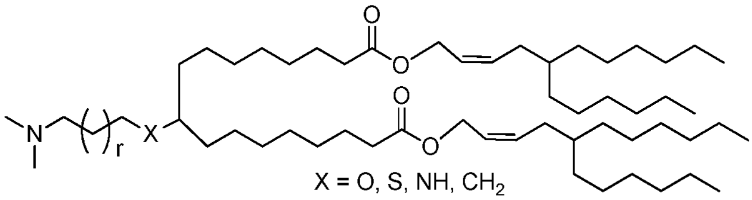
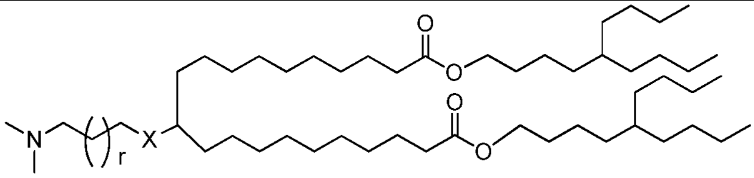


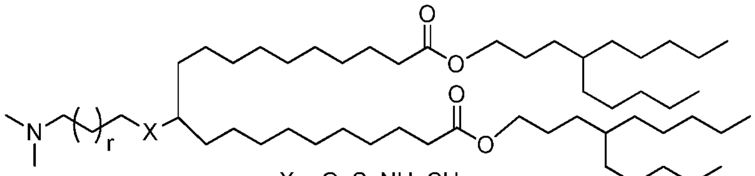
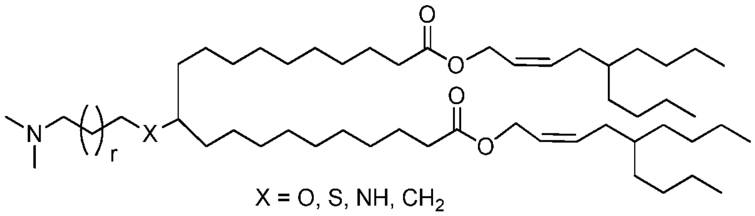
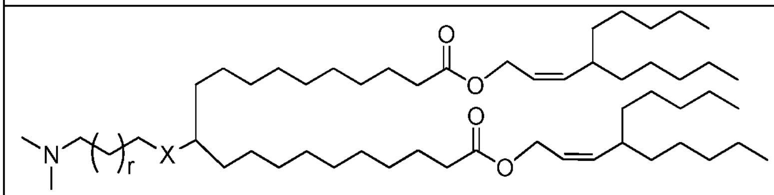
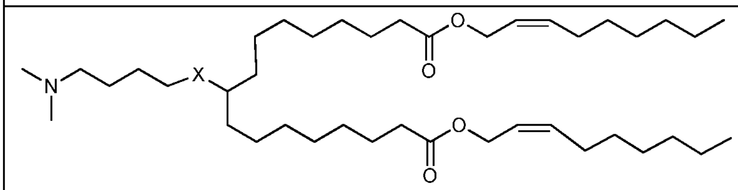
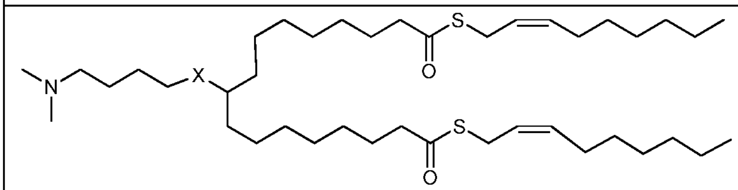









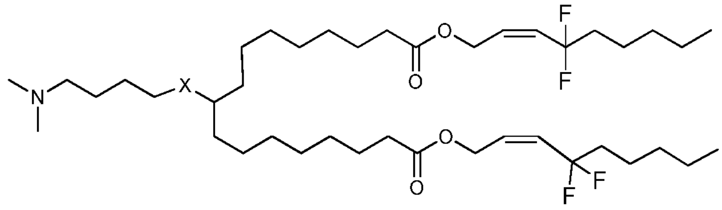






 r = 0, 1, or 2


z



 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2

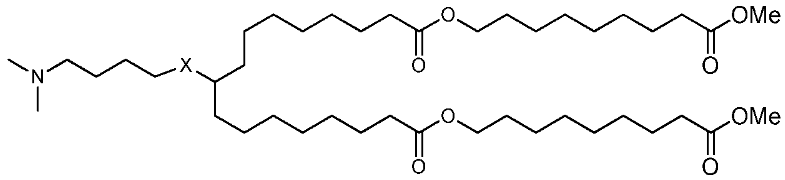
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2
 X = O, S, NR, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2

 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2

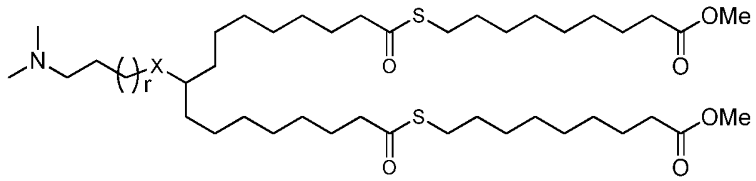
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2
 X = O, S, NH, CH_2



X = O, S, NH, CH_2

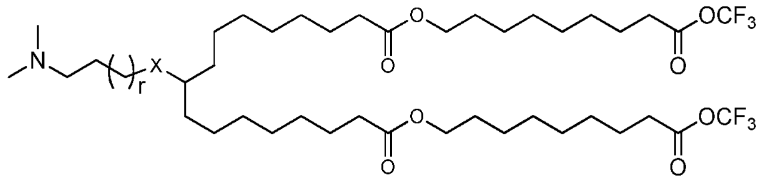


X = O, S, NH, CH2



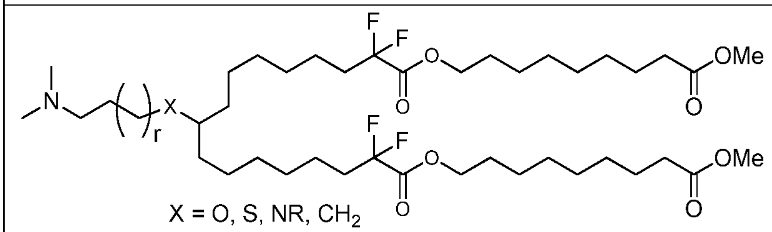
X = O, S, NH, CH_2

r = 0, 1, or 2



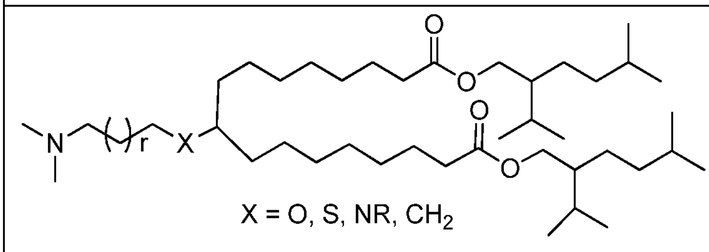
X = O, S, NR, CH_2

r = 0, 1, or 2

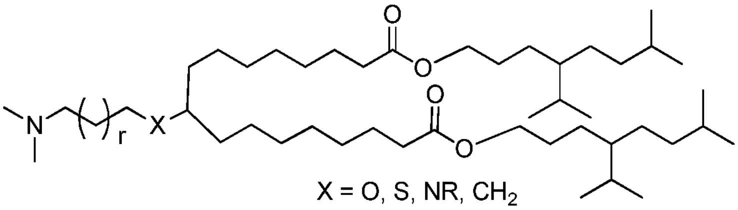
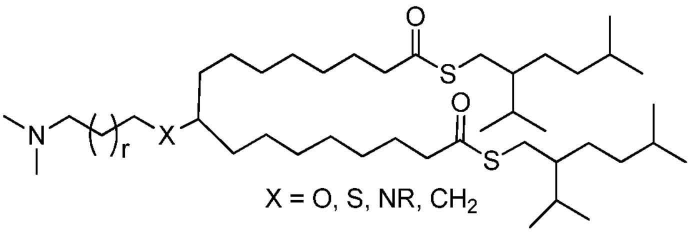
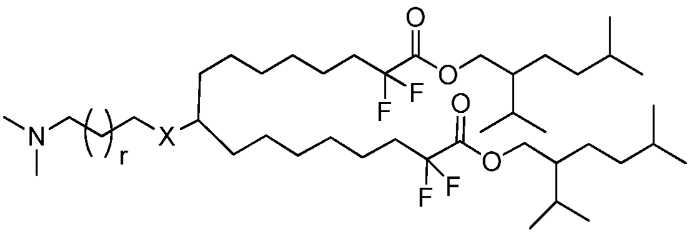
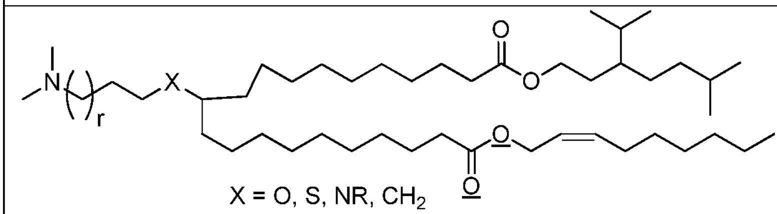
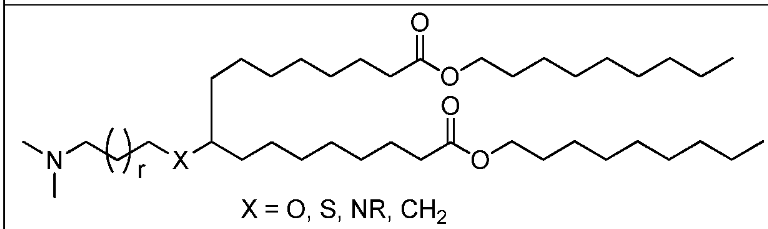


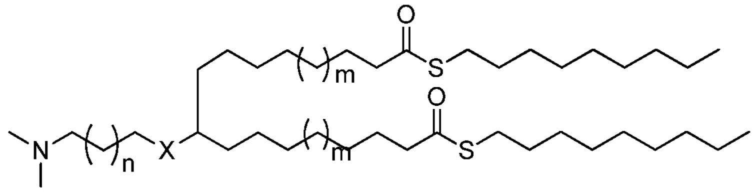
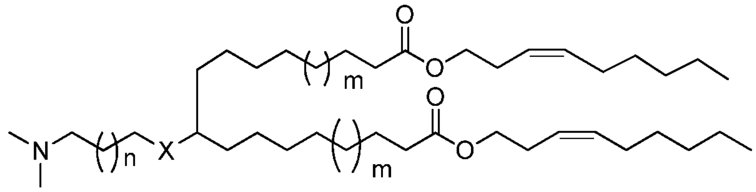
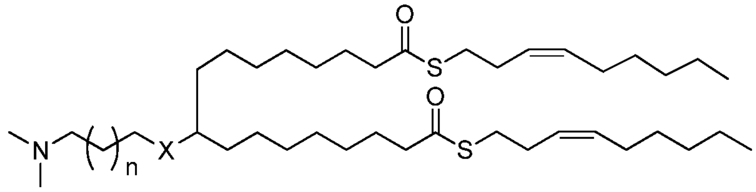
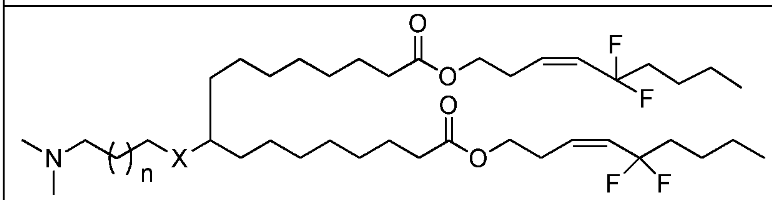
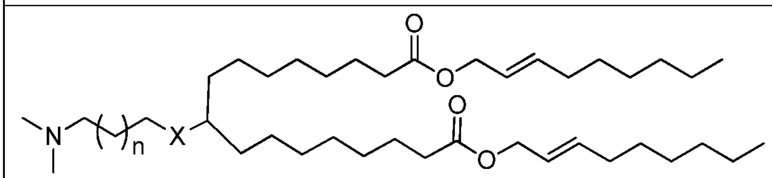
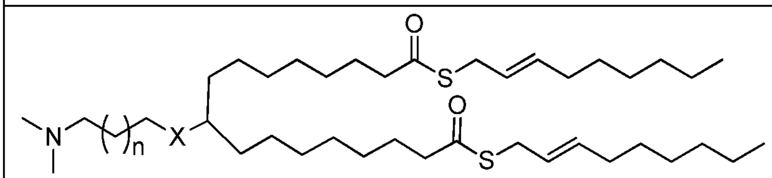
X = O, S, NR, CH_2

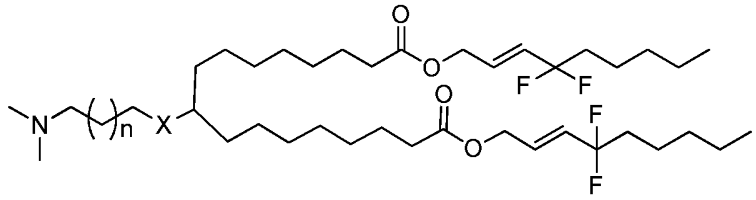
r = 0, 1, or 2



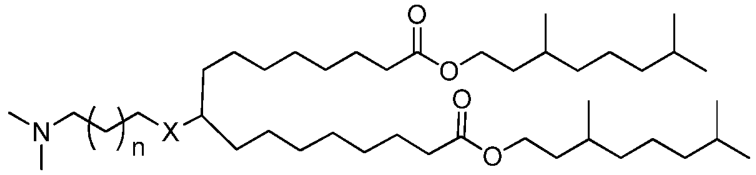
X = O, S, NR, CH_2

r = 0, 1, or 2
 r = 0, 1, or 2
 r = 0, 1, or 2
 r = 0, 1, or 2
 r = 0, 1, or 2
 r = 0, 1, or 2

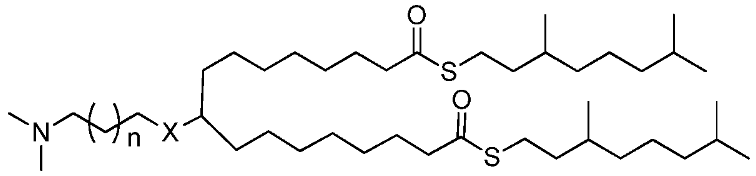
 m = 0-5, n = 0, 1, or 2
 X = O, S, NR, CH_2 m = 0-5, n = 0, 1, or 2
 n = 0, 1, or 2
 n = 0, 1, or 2
 n = 0, 1, or 2
 n = 0, 1, or 2



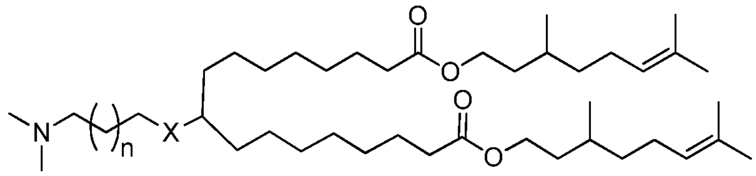
n = 0, 1, or 2



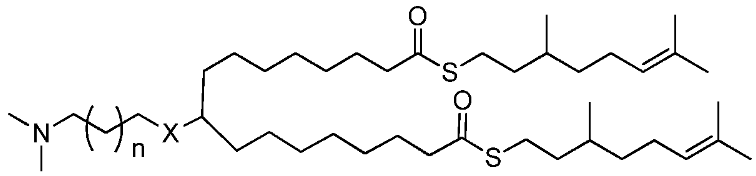
n = 0, 1, or 2



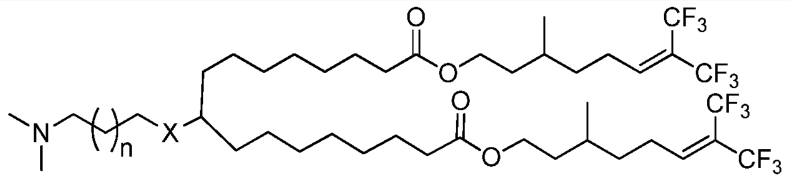
n = 0, 1, or 2



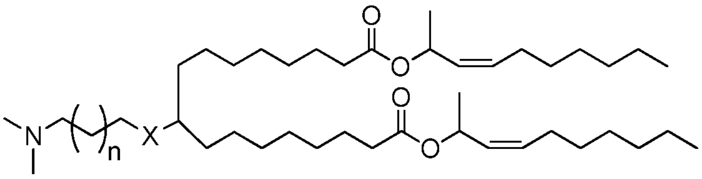
n = 0, 1, or 2



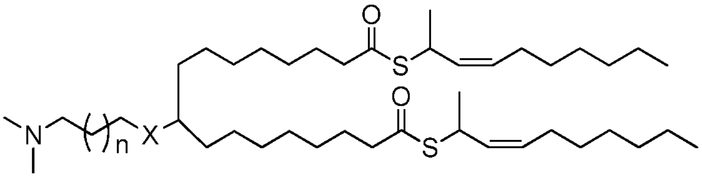
n = 0, 1, or 2



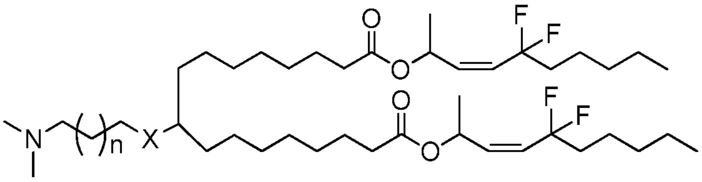
n = 0, 1, or 2



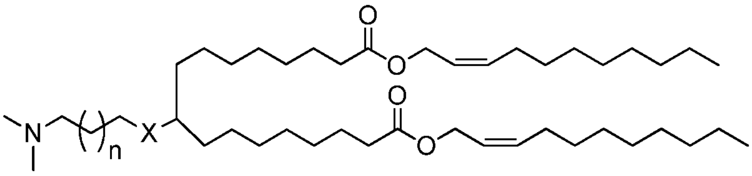
n = 0, 1, or 2



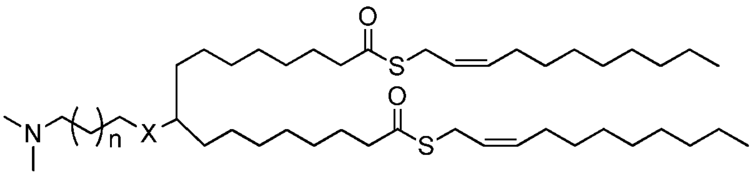
n = 0, 1, or 2



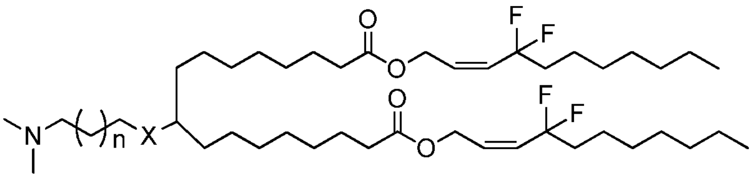
n = 0, 1, or 2



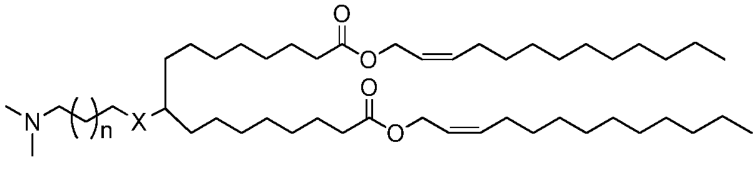
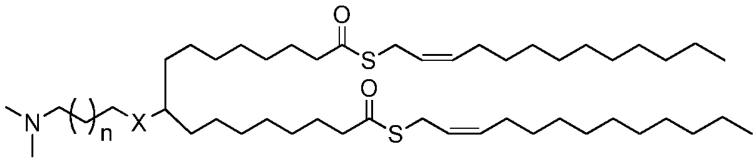
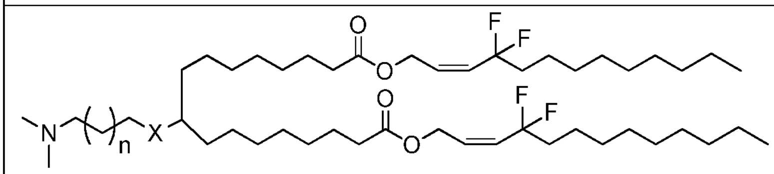
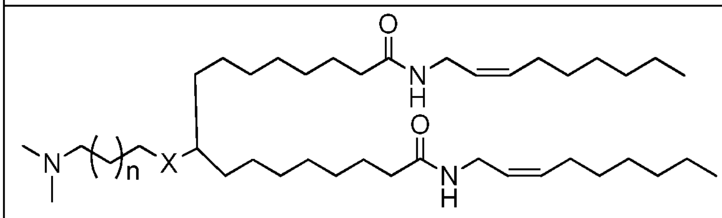
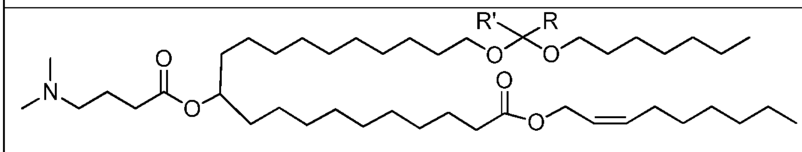
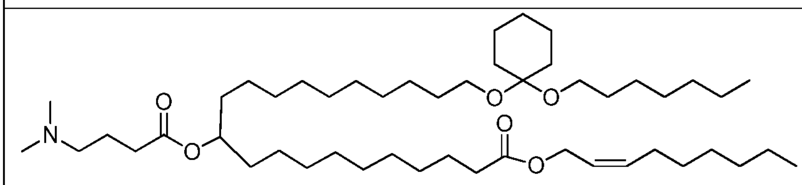
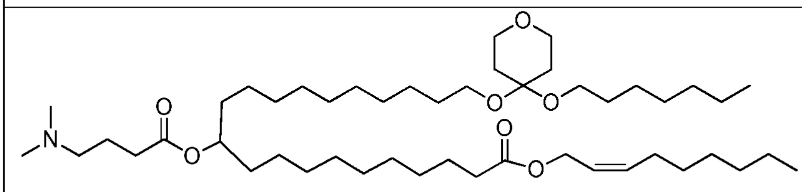
n = 0, 1, or 2

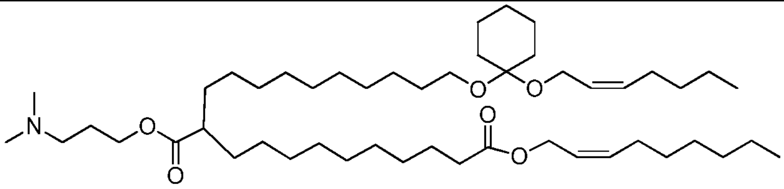
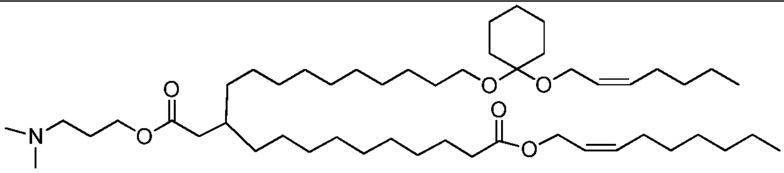
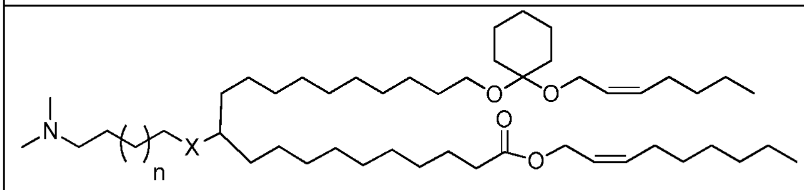
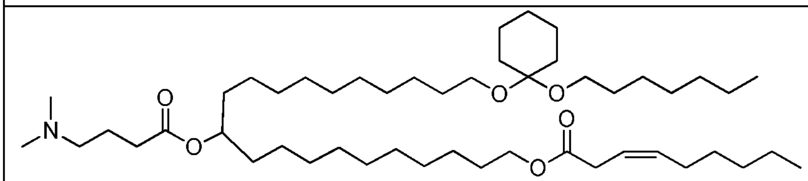
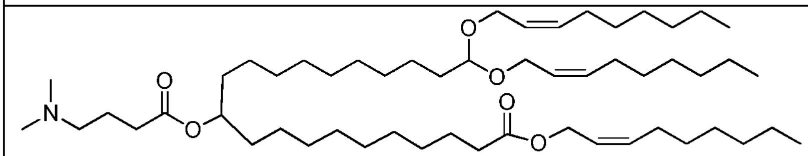
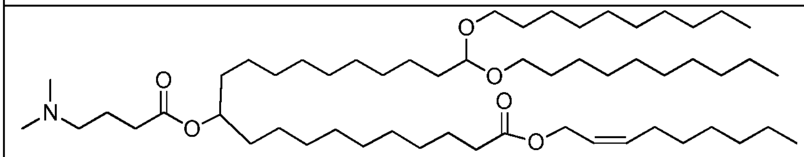
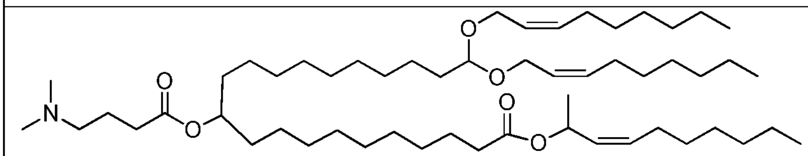
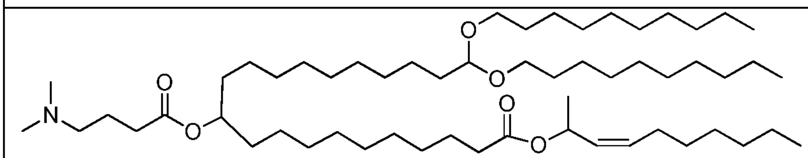


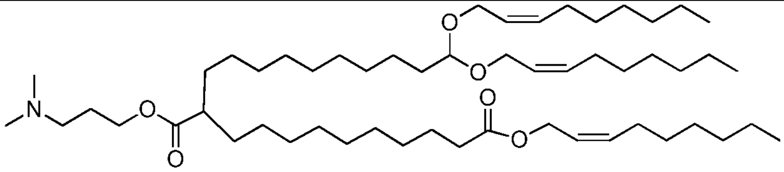
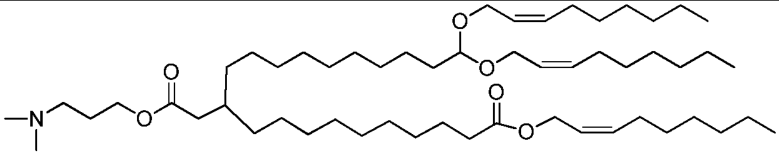
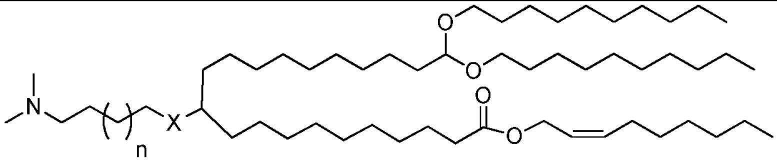
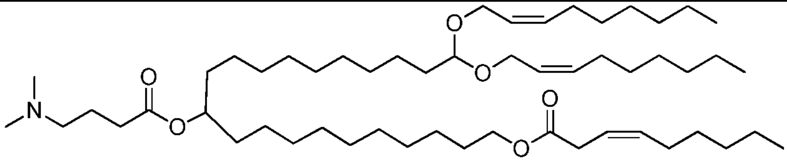
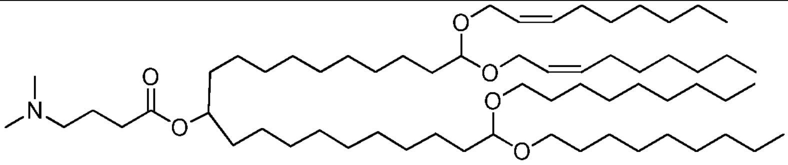
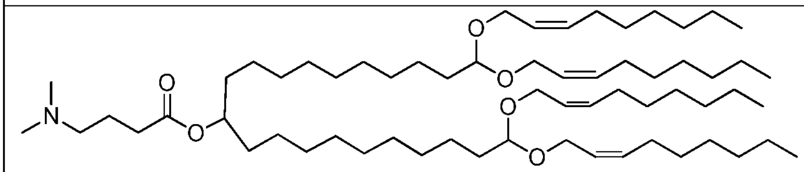
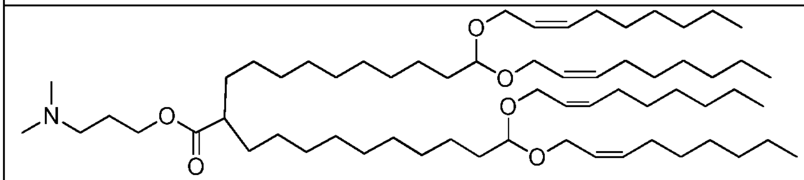
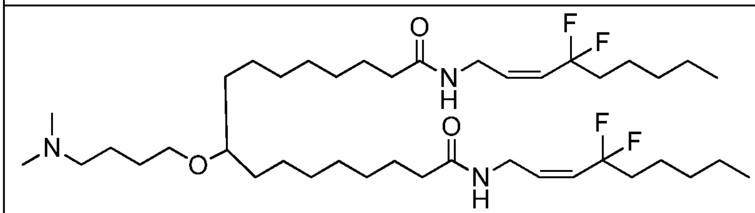
n = 0, 1, or 2

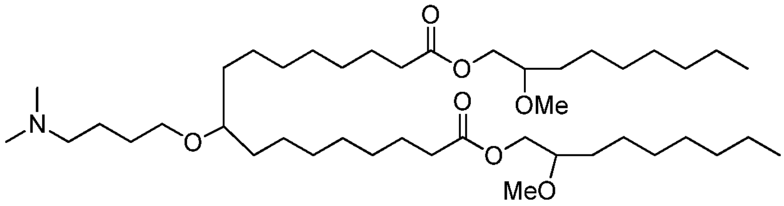
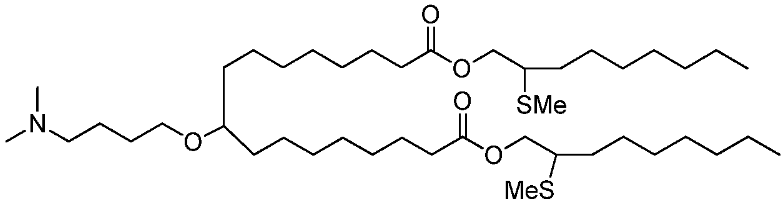
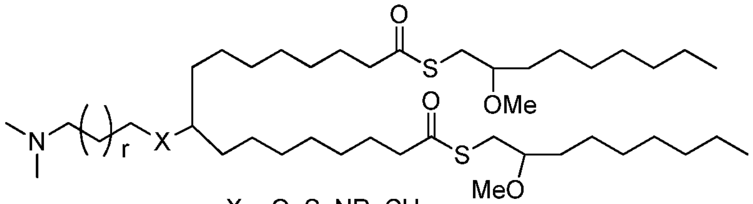
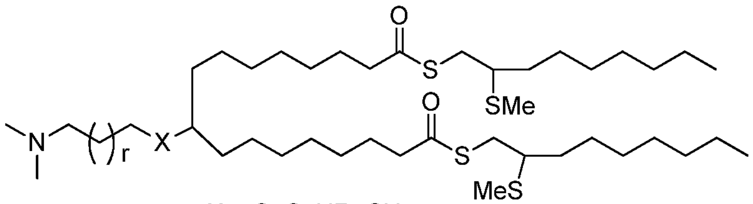
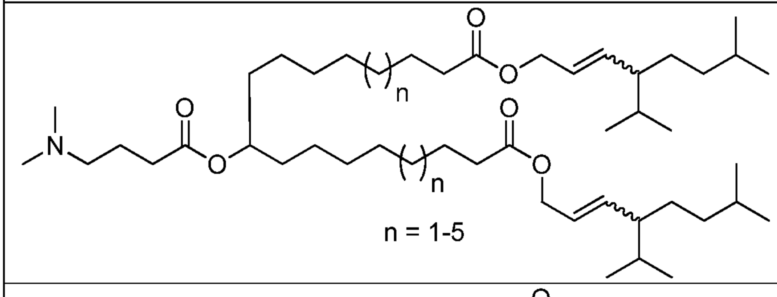
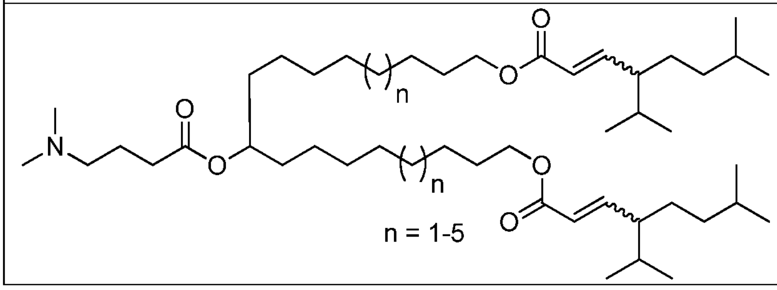


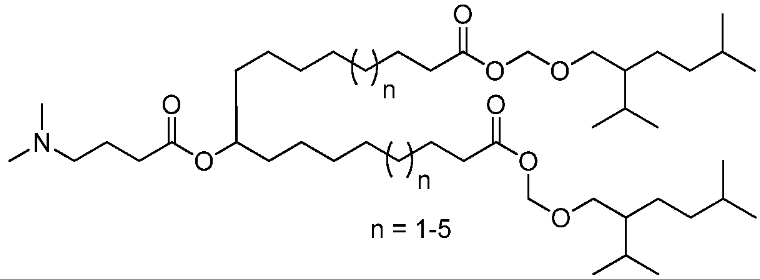
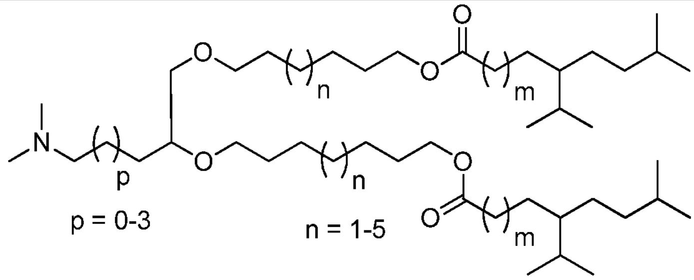
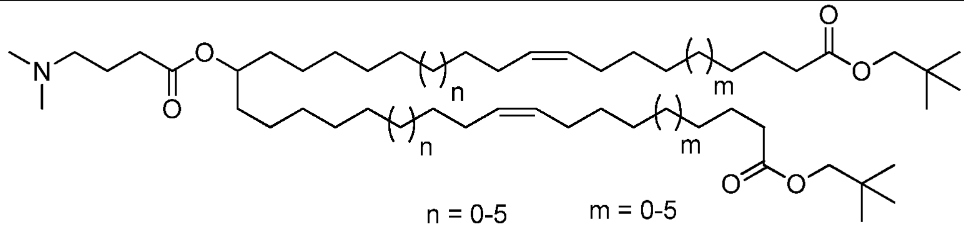
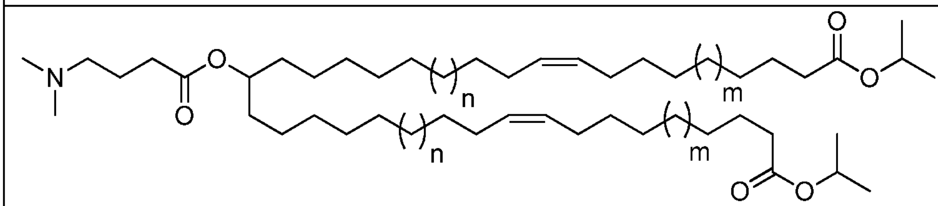
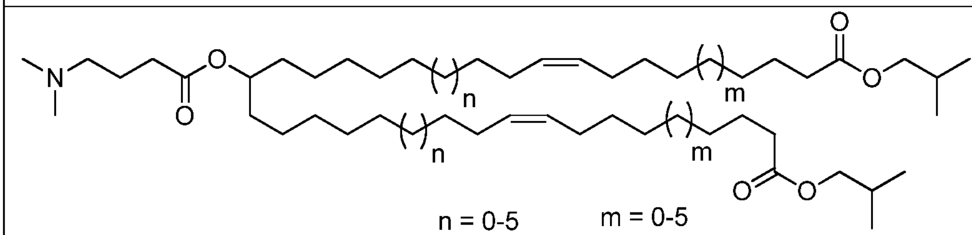
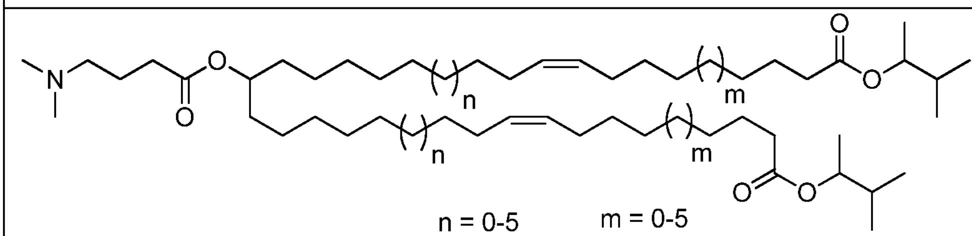
n = 0, 1, or 2

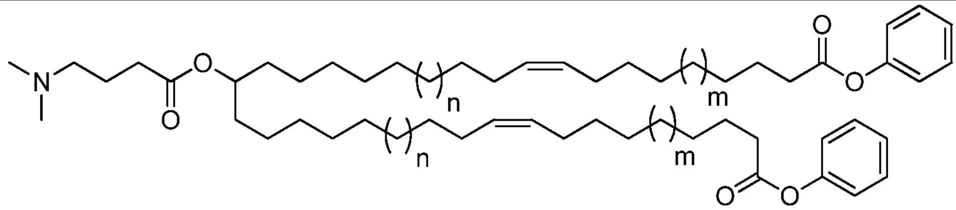
 n = 0, 1, or 2
 n = 0, 1, or 2
 n = 0, 1, or 2
 n = 0, 1, or 2
 R, R' = CH_3, Cylcopentyl etc





 X = O, S, NR, CH_2, COO, NHCOO, OCONH n = 0, 1, or 2






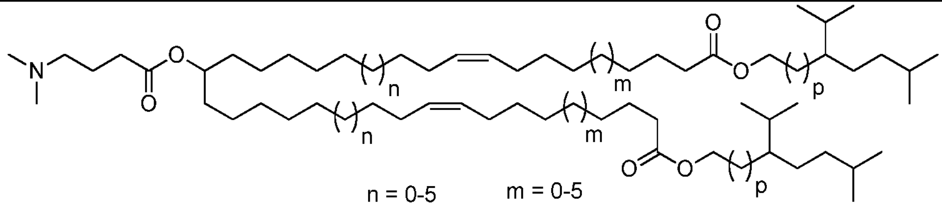


 X = O, S, NR, CH_2, COO, NHCOO, OCONH n = 0, 1, or 2








 X = O, S, NR, CH_2 r = 0, 1, or 2
 X = O, S, NR, CH_2 r = 0, 1, or 2
 n = 1-5
 n = 1-5

 n = 1-5
 p = 0-3 n = 1-5 m = 0-3
 n = 0-5 m = 0-5
 n = 0-5 m = 0-5
 n = 0-5 m = 0-5
 n = 0-5 m = 0-5

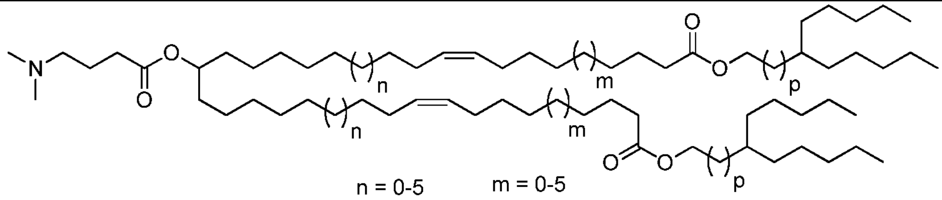


n = 0-5 m = 0-5



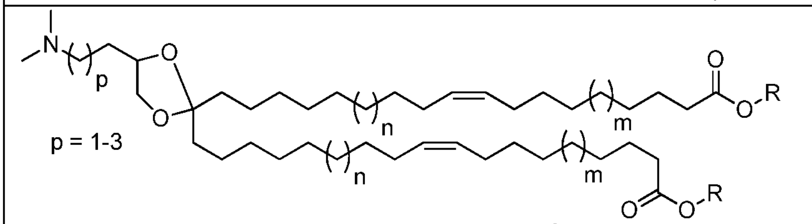
n = 0-5 m = 0-5

p = 0-3



n = 0-5 m = 0-5

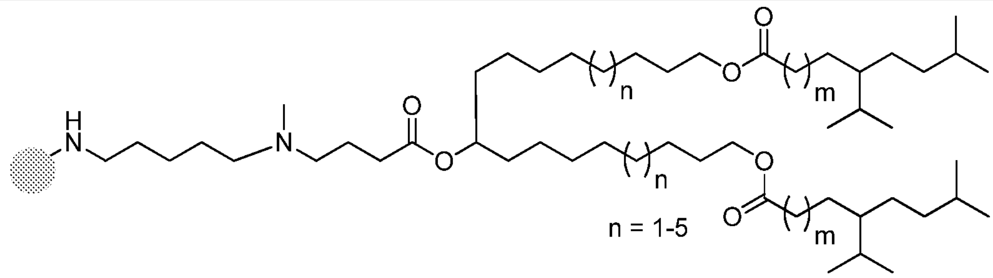
p = 0-3



p = 1-3

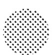
n = 0-5 m = 0-5

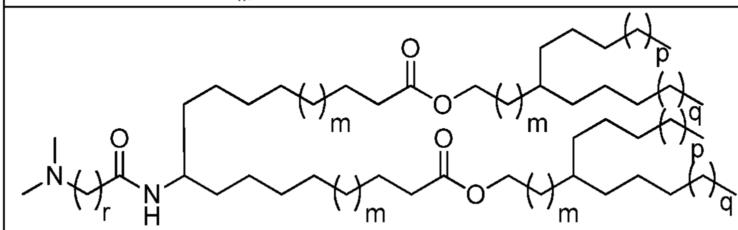
R = alkyl, substituted alkyl, aryl



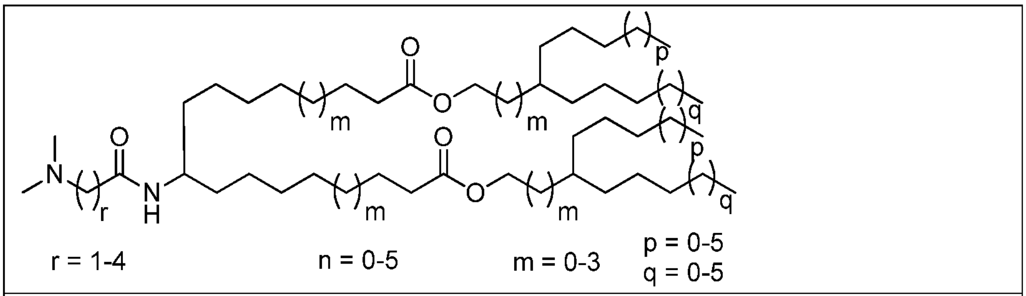
n = 1-5

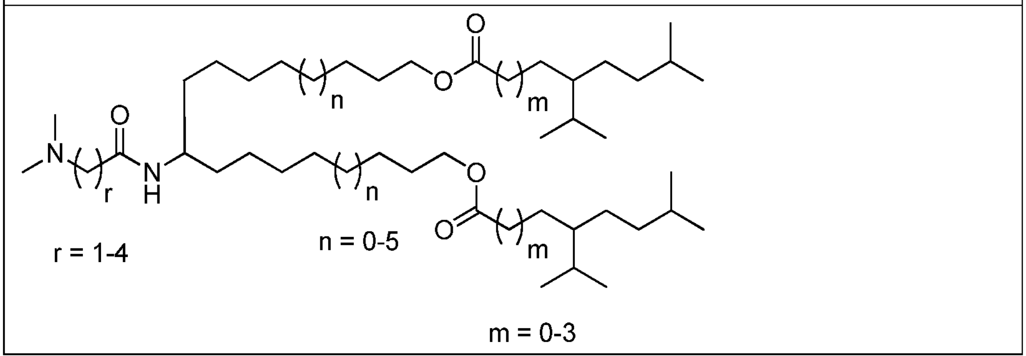
m = 0-3

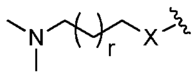
 = Bodipy, Alexa-647 or other label (e.g., other fluorescent label)

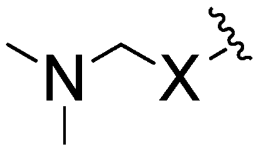


r = 1-4 n = 0-5 m = 0-3 p = 0-5 q = 0-5





Alternatively, for the compounds above having a head of the formula  (where X can be, for example, -C(O)O-), the head can have one methylene unit between the X group (or other functional group) and nitrogen atom. For example, the head can be:



Cationic lipids include those having alternative fatty acid groups and other dialkylamino groups than those shown, including those in which the alkyl substituents are different (*e.g.*, N-ethyl-N-methylamino-, and N-propyl-N-ethylamino-).

In certain embodiments, the cationic lipids have at least one protonatable or deprotonatable group, such that the lipid is positively charged at a pH at or below physiological pH (e.g. pH 7.4), and neutral at a second pH, preferably at or above physiological pH. Such lipids are also referred to as cationic lipids. It will, of course, be understood that the addition or removal of protons as a function of pH is an equilibrium process, and that the reference to a charged or a neutral lipid refers to the nature of the predominant species and does not require that all of the lipid be present in the charged or neutral form. The lipids can have more than one protonatable or deprotonatable group, or can be zwiterrionic.

In certain embodiments, protonatable lipids (i.e., cationic lipids) have a pK_a of the protonatable group in the range of about 4 to about 11. For example, the lipids can have a pK_a of about 4 to about 7, e.g., from about 5 to about 7, such as from about 5.5 to about 6.8, when incorporated into lipid particles. Such lipids may be cationic at a lower pH formulation stage, while particles will be largely (though not completely) surface neutralized at physiological pH around pH 7.4.

In particular embodiments, the lipids are charged lipids. As used herein, the term "charged lipid" includes, but is not limited to, those lipids having one or two fatty acyl or fatty alkyl chains and a quaternary amino head group. The quaternary amine carries a permanent positive charge. The head group can optionally include an ionizable group, such as a primary, secondary, or tertiary amine that may be protonated at physiological pH. The presence of the quaternary amine can alter the pKa of the ionizable group relative to the pKa of the group in a structurally similar compound that lacks the quaternary amine (e.g., the quaternary amine is replaced by a tertiary amine).

Included in the instant invention is the free form of the cationic lipids described herein, as well as pharmaceutically acceptable salts and stereoisomers thereof. The cationic lipid can be a protonated salt of the amine cationic lipid. The term "free form" refers to the amine cationic lipids in non-salt form. The free form may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaOH, potassium carbonate, ammonia and sodium bicarbonate.

The pharmaceutically acceptable salts of the instant cationic lipids can be synthesized from the cationic lipids of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic cationic lipids are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

Thus, pharmaceutically acceptable salts of the cationic lipids of this invention include non-toxic salts of the cationic lipids of this invention as formed by reacting a basic instant cationic lipids with an inorganic or organic acid. For example, non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and trifluoroacetic (TFA).

When the cationic lipids of the present invention are acidic, suitable "pharmaceutically acceptable salts" refers to salts prepared form pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, and zinc. In one embodiment, the base is selected from ammonium, calcium, magnesium, potassium and sodium. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine caffeine, choline, N,N^1-dibenzylethylenediamine, diethylamin, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine tripropylamine, and tromethamine.

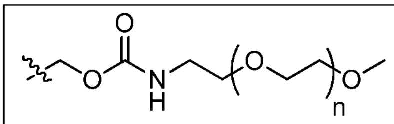
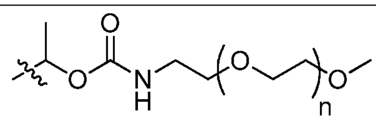
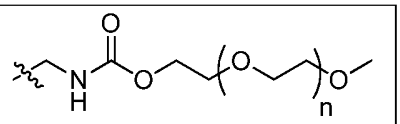
It will also be noted that the cationic lipids of the present invention may potentially be internal salts or zwitterions, since under physiological conditions a deprotonated acidic moiety in the compound, such as a carboxyl group, may be anionic, and this electronic charge might then be balanced off internally against the cationic charge of a protonated or alkylated basic moiety, such as a quaternary nitrogen atom.

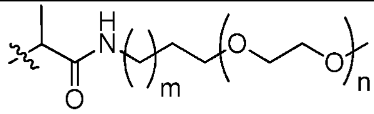
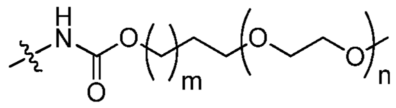
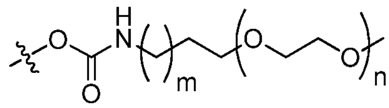
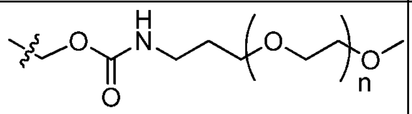
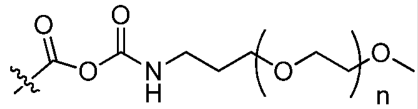
One or more additional cationic lipids, which carry a net positive charge at about physiological pH, in addition to those specifically described above, may also be included in the lipid particles and compositions described herein. Such cationic lipids include, but are not limited to N,N-dioleyl-N,N-dimethylammonium chloride ("DODAC"); N-(2,3-dioleyloxy)propyl-N,N-N-triethylammonium chloride ("DOTMA"); N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP"); 1,2-Dioleyloxy-3-trimethylaminopropane chloride salt ("DOTAP.Cl"); 3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol"), N-(1-(2,3-dioleyloxy)propyl)-N-2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoracetate ("DOSPA"), dioctadecylamidoglycyl carboxyspermine ("DOGS"), 1,2-dileoyl-sn-3-phosphoethanolamine ("DOPE"), 1,2-dioleoyl-3-dimethylammonium propane ("DODAP"), N, N-dimethyl-2,3-dioleyloxy)propylamine ("DODMA"), and N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE"). Additionally, a number of commercial preparations of cationic lipids can be used, such as, *e.g.*, LIPOFECTIN (including DOTMA and DOPE, available from GIBCO/BRL), and LIPOFECTAMINE (comprising DOSPA and DOPE, available from GIBCO/BRL).

PEG Lipids

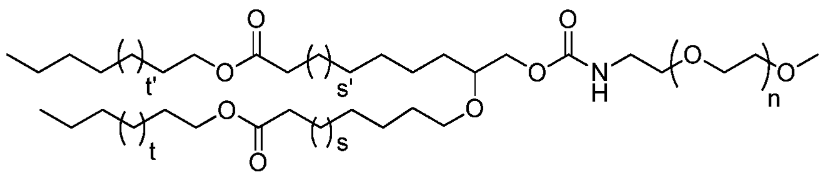
Suitable head groups for the PEG lipids include, but are not limited to those shown in Table 3 below.

Table 3

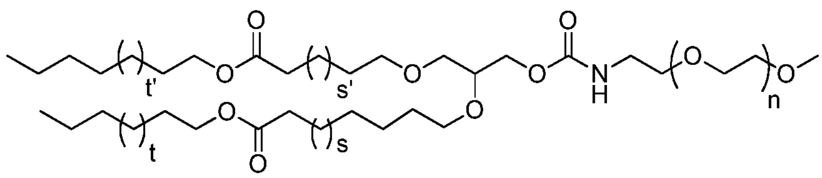
		

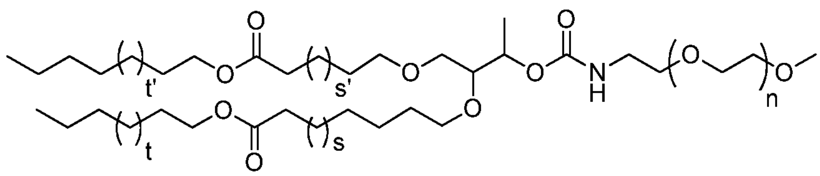
Representative PEG lipids include, but are not limited to:

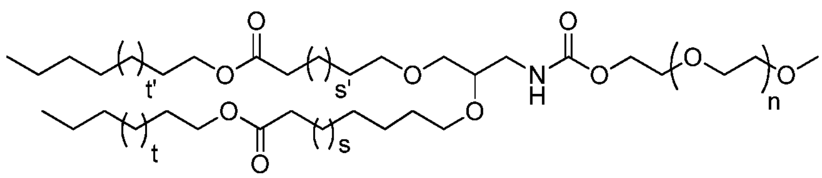


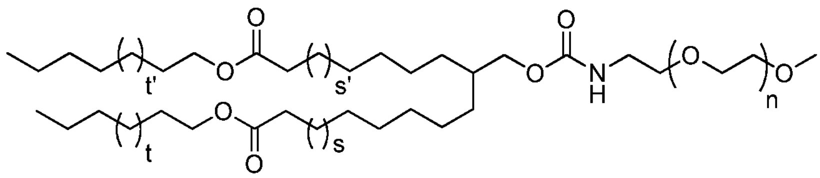
,

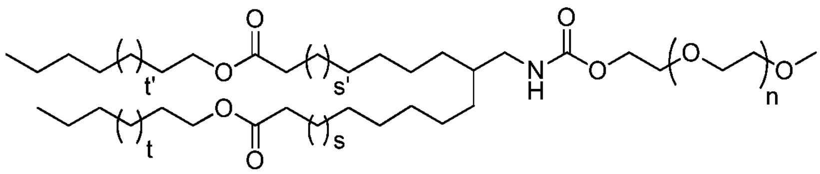


,

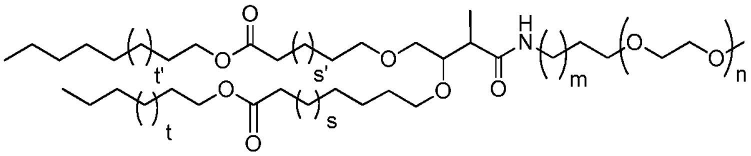




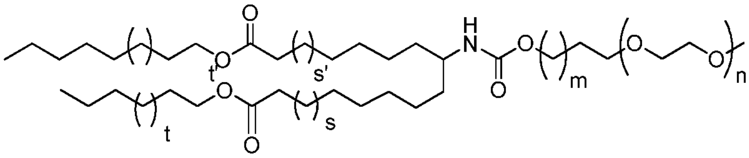


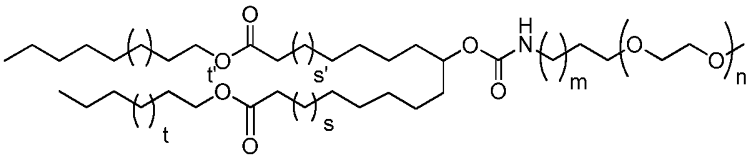


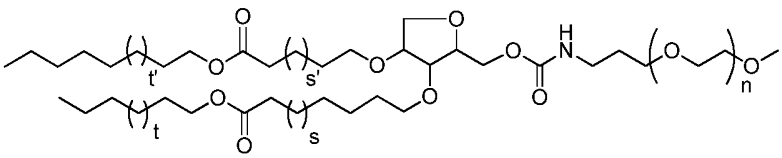
,



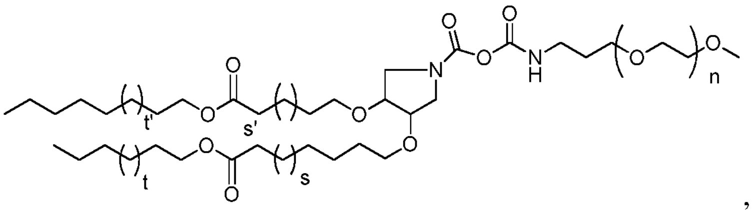
,







,



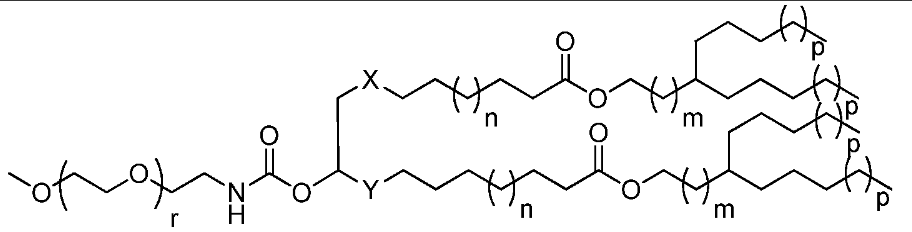
,

wherein

n is an integer from 10 to 100 (e.g. 20-50 or 40-50);

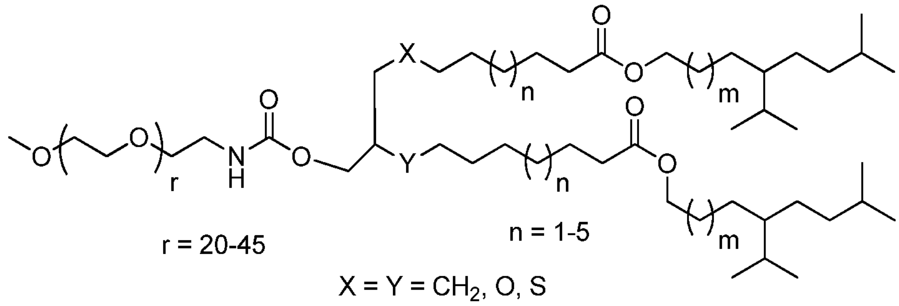
s, s', t and t' are independently 0, 1, 2, 3, 4, 5, 6 or 7; and m is 1, 2, 3, 4, 5, or 6.

Other representative PEG lipids include, but are not limited to:



r = 20-45 n = 1-5 m = 0-3 p = 1-5

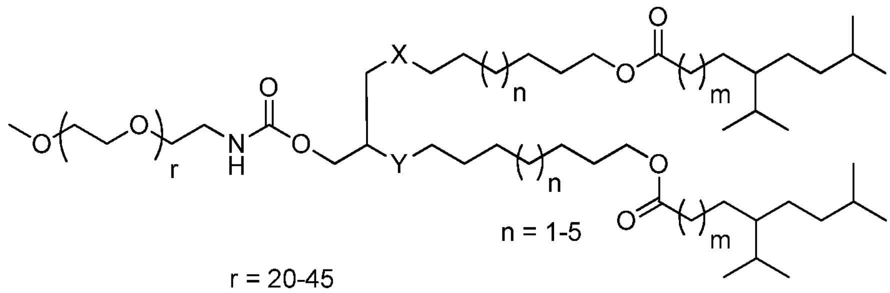
X = Y = CH_2, O, S



r = 20-45 n = 1-5

X = Y = CH_2, O, S

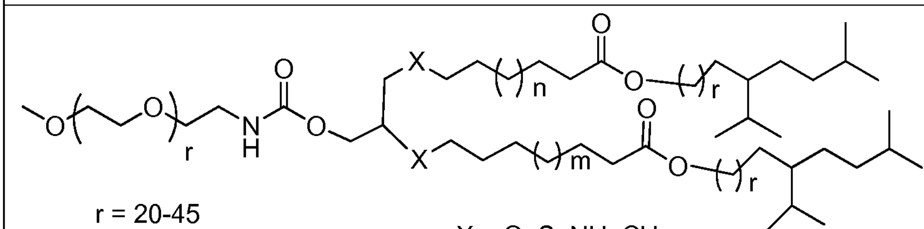
m = 0-3



n = 1-5

r = 20-45

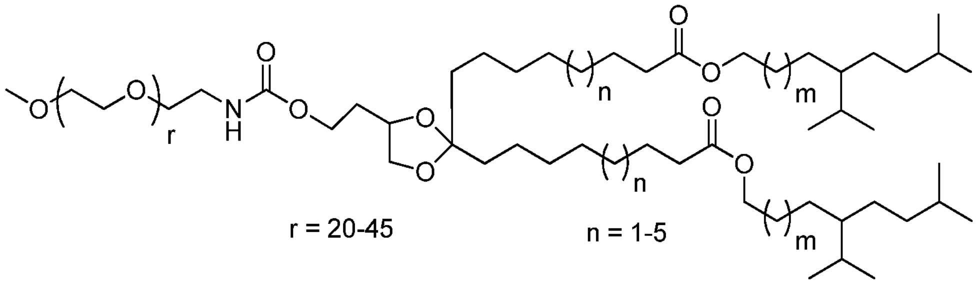
X = Y = CH_2, O, S m = 0-3



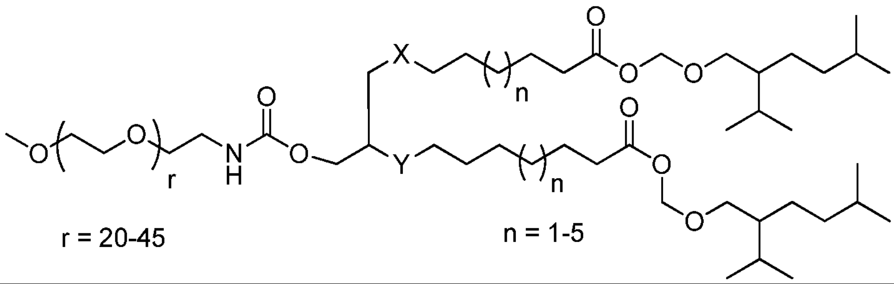
r = 20-45

X = O, S, NH, CH_2

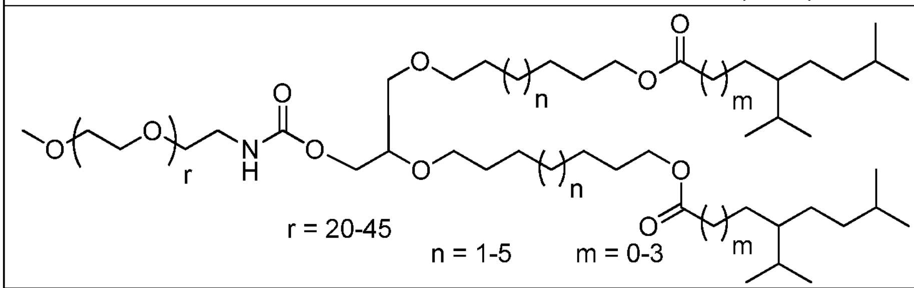
m = n = r = 0-10



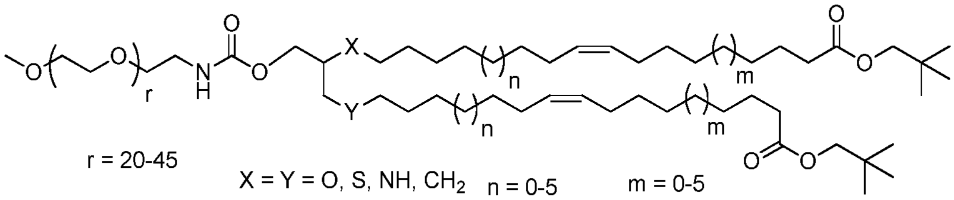
r = 20-45 n = 1-5 m = 0-3



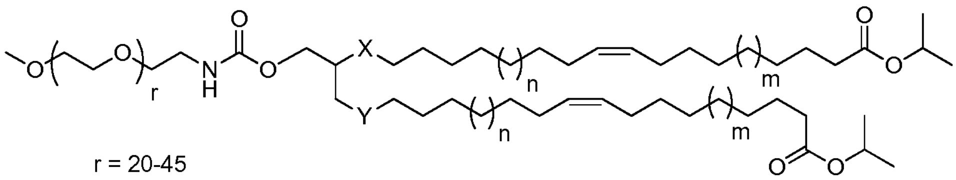
r = 20-45 n = 1-5



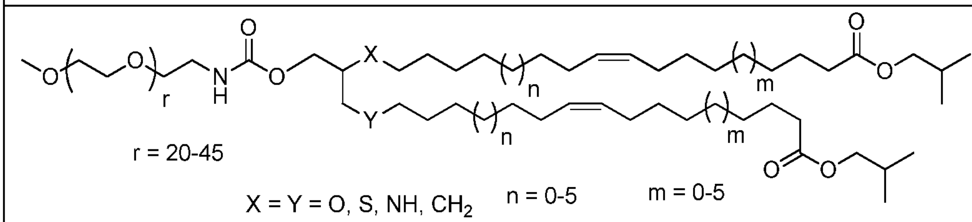
r = 20-45 n = 1-5 m = 0-3



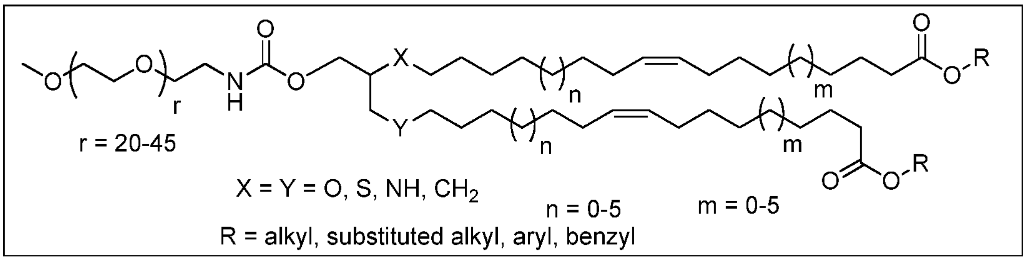
r = 20-45 X = Y = O, S, NH, CH_2 n = 0-5 m = 0-5



r = 20-45 X = Y = O, S, NH, CH_2 n = 0-5 m = 0-5



r = 20-45 X = Y = O, S, NH, CH_2 n = 0-5 m = 0-5



The Other Lipid Components

The lipid particles and compositions described herein may also include one or more neutral lipids. Neutral lipids, when present, can be any of a number of lipid species which exist either in an uncharged or neutral zwitterionic form at physiological pH. Such lipids include, for example, diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, dihydrosphingomyelin, cephalin, and cerebrosides. In one embodiment, the neutral lipid component is a lipid having two acyl groups (*e.g.,* diacylphosphatidylcholine and diacylphosphatidylethanolamine). In one embodiment, the neutral lipid contains saturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20}. In another embodiment, the neutral lipid includes mono or diunsaturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20}. Suitable neutral lipids include, but are not limited to, DSPC, DPPC, POPC, DOPE, DSPC, and SM.

The lipid particles and compositions described herein may also include one or more lipids capable of reducing aggregation. Examples of lipids that reduce aggregation of particles during formation include polyethylene glycol (PEG)-modified lipids (PEG lipids, such as PEG-DMG and PEG-DMA), monosialoganglioside Gm1, and polyamide oligomers ("PAO") such as (described in U.S. Patent No. 6,320,017, which is incorporated by reference in its entirety). Suitable PEG lipids include, but are not limited to, PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (*e.g.,* PEG-CerC14 or PEG-CerC20) (such as those described in U.S. Patent No. 5,820,873, incorporated herein by reference), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines, PEG-modified diacylglycerols and dialkylglycerols, mPEG (mw2000)-diastearoylphosphatidylethanolamine (PEG-DSPE).

The lipid particles and compositions may include a sterol, such as cholesterol.

Lipid Particles

In a further aspect, the present invent relates to lipid particles that include one or more of the cationic lipids described herein. In one embodiment, the lipid particle includes one or more compounds of formula I-VII.

Lipid particles include, but are not limited to, liposomes. As used herein, a liposome is a structure having lipid-containing membranes enclosing an aqueous interior.

Another embodiment is a nucleic acid-lipid particle (e.g., a SNALP) comprising a cationic lipid of the present invention, a non-cationic lipid (such as a neutral lipid), optionally a PEG-lipid conjugate (such as the lipids for reducing aggregation of lipid particles discussed herein), optionally a sterol (e.g., cholesterol), and a nucleic acid. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP represents a particle made from lipids, wherein the nucleic acid (e.g., an interfering RNA) is encapsulated within the lipids. In certain instances, SNALPs are useful for systemic applications, as they can exhibit extended circulation lifetimes following intravenous (i.v.) injection, they can accumulate at distal sites (e.g., sites physically separated from the administration site), and they can mediate silencing of target gene expression at these distal sites. The nucleic acid may be complexed with a condensing agent and encapsulated within a SNALP as set forth in International Publication No. WO 00/03683, the disclosure of which is herein incorporated by reference in its entirety.

For example, the lipid particle may include a cationic lipid, a fusion-promoting lipid (e.g., DPPC), a neutral lipid, cholesterol, and a PEG-modified lipid. In one embodiment, the lipid particle includes the above lipid mixture in molar ratios of about 20-70% cationic lipid: 0.1-50% fusion promoting lipid: 5-45% neutral lipid: 20-55% cholesterol: 0.5-15% PEG-modified lipid (based upon 100% total moles of lipid in the lipid particle).

In another embodiment of the lipid particle, the cationic lipid is present in a mole percentage of about 20% and about 60%; the neutral lipid is present in a mole percentage of about 5% to about 25%; the sterol is present in a mole percentage of about 25% to about 55%; and the PEG lipid is PEG-DMA, PEG-DMG, or a combination thereof, and is present in a mole percentage of about 0.5% to about 15% (based upon 100% total moles of lipid in the lipid particle).

In particular embodiments, the molar lipid ratio, with regard to mol% cationic lipid/DSPC/Chol/PEG-DMG or PEG-DMA) is approximately 40/10/40/10, 35/15/40/10 or 52/13/30/5. This mixture may be further combined with a fusion-promoting lipid in a molar ratio of 0.1-50%, 0.1-50%, 0.5-50%, 1-50%, 5%-45%, 10%-40%, or 15%-35%. In other words, when a 40/10/40/10 mixture of lipid/DSPC/Chol/PEG-DMG or PEG-DMA is combined with a fusion-promoting peptide in a molar ratio of 50%, the resulting lipid particles can have a total molar ratio of (mol% cationic lipid/DSPC/Chol/PEG-DMG or PEG-DMA/fusion-promoting peptide) 20/5/20/5/50. In another embodiment, the neutral lipid, DSPC, in these compositions is replaced with POPC, DPPC, DOPE or SM.

In one embodiment, the lipid particles comprise a cationic lipid of the present invention, a neutral lipid, a sterol and a PEG-modified lipid. In one embodiment, the lipid particles include from about 25% to about 75% on a molar basis of cationic lipid, e.g., from about 35 to about 65%, from about 45 to about 65%, about 60%, about 57.5%, about 57.1%, about 50% or about 40% on a molar basis. In one embodiment, the lipid particles include from about 0% to about 15% on a molar basis of the neutral lipid, e.g., from about 3 to about 12%, from about 5 to about 10%, about 15%, about 10%, about 7.5%, about 7.1% or about 0% on a molar basis. In one embodiment, the neutral lipid is DPPC. In one embodiment, the neutral lipid is DSPC. In one embodiment, the formulation includes from about 5% to about 50% on a molar basis of the sterol, e.g., about 15 to about 45%, about 20 to about 40%, about 48%, about 40%, about 38.5%, about 35%, about 34.4%, about 31.5% or about 31% on a molar basis. In one embodiment, the sterol is cholesterol.

The lipid particles described herein may further include one or more therapeutic agents. In a preferred embodiment, the lipid particles include a nucleic acid (e.g., an oligonucleotide), such as siRNA or miRNA.

In one embodiment, the lipid particles include from about 0.1% to about 20% on a molar basis of the PEG-modified lipid, e.g., about 0.5 to about 10%, about 0.5 to about 5%, about 10%, about 5%, about 3.5%, about 1.5%, about 0.5%, or about 0.3% on a molar basis. In one embodiment, the PEG-modified lipid is PEG- DMG. In one embodiment, the PEG-modified lipid is PEG-c-DMA. In one embodiment, the lipid particles include 25-75% of cationic lipid, 0.5-15% of the neutral lipid, 5-50% of the sterol, and 0.5- 20% of the PEG-modified lipid on a molar basis.

In one embodiment, the lipid particles include 35-65% of cationic lipid, 3-12% of the neutral lipid, 15-45% of the sterol, and 0.5- 10% of the PEG-modified lipid on a molar basis. In one embodiment, the lipid particles include 45-65% of cationic lipid, 5-10% of the neutral lipid, 25-40% of the sterol, and 0.5- 5% of the PEG-modified lipid on a molar basis. In one embodiment, the PEG modified lipid comprises a PEG molecule of an average molecular weight of 2,000 Da. In one embodiment, the PEG modified lipid is PEG-distyryl glycerol (PEG-DSG).

In one embodiment, the ratio of lipid:siRNA is at least about 0.5:1, at least about 1:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 11:1 or at least about 33:1. In one embodiment, the ratio of lipid: siRNA ratio is between about 1:1 to about 35:1, about 3:1 to about 15:1, about 4:1 to about 15:1, or about 5:1 to about 13:1. In one embodiment, the ratio of lipid:siRNA ratio is between about 0.5:1 to about 12:1.

In one embodiment, the lipid particles are nanoparticles. In additional embodiments, the lipid particles have a mean diameter size of from about 50 nm to about 300 nm, such as from about 50 nm to about 250 nm, for example, from about 50 nm to about 200 nm.

In one embodiment, a lipid particle containing a cationic lipid of any of the embodiments described herein has an *in vivo* half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 3 hours, such as less than about 2.5 hours, less than about 2 hours, less than about 1.5 hours, less than about 1 hour, less than about 0.5 hour or less than about 0.25 hours.

In another embodiment, a lipid particle containing a cationic lipid of any of the embodiments described herein has an *in vivo* half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 10 % (e.g., less than about 7.5%, less than about 5%, less than about 2.5%) of that for the same cationic lipid without the biodegrable group or groups.

Additional Components

The lipid particles and compositions described herein can further include one or more antioxidants. The antioxidant stabilizes the lipid particle and prevents, decreases, and/or inhibits degradation of the cationic lipid and/or active agent present in the lipid particles. The antioxidant can be a hydrophilic antioxidant, a lipophilic antioxidant, a metal chelator, a primary antioxidant, a secondary antioxidant, salts thereof, and mixtures thereof. In certain embodiments, the antioxidant comprises a metal chelator such as EDTA or salts thereof, alone or in combination with one, two, three, four, five, six, seven, eight, or more additional antioxidants such as primary antioxidants, secondary antioxidants, or other metal chelators. In one preferred embodiment, the antioxidant comprises a metal chelator such as EDTA or salts thereof in a mixture with one or more primary antioxidants and/or secondary antioxidants. For example, the antioxidant may comprise a mixture of EDTA or a salt thereof, a primary antioxidant such as a-tocopherol or a salt thereof, and a secondary antioxidant such as ascorbyl palmitate or a salt thereof. In one embodiment, the antioxidant comprises at least about 100 mM citrate or a salt thereof. Examples of antioxidants include, but are not limited to, hydrophilic antioxidants, lipophilic antioxidants, and mixtures thereof. Non-limiting examples of hydrophilic antioxidants include chelating agents (e.g., metal chelators) such as ethylenediaminetetraacetic acid (EDTA), citrate, ethylene glycol tetraacetic acid (EGTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), diethylene triamine pentaacetic acid (DTPA), 2,3-dimercapto-l-propanesulfonic acid (DMPS), dimercaptosuccinic acid (DMSA), cc-lipoic acid, salicylaldehyde isonicotinoyl hydrazone (SIH), hexyl thioethylamine hydrochloride (HTA), desferrioxamine, salts thereof, and mixtures thereof. Additional hydrophilic antioxidants include ascorbic acid, cysteine, glutathione, dihydrolipoic acid, 2- mercaptoethane sulfonic acid, 2-mercaptobenzimidazole sulfonic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, sodium metabisulfite, salts thereof, and mixtures thereof. Non-limiting examples of lipophilic antioxidants include vitamin E isomers such as α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δtocotrienols; polyphenols such as 2-tert-butyl-4-methyl phenol, 2-fert-butyl-5-methyl phenol, and 2-tert-butyl-6-methyl phenol; butylated hydroxyanisole (BHA) (e.g., 2-teri-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole); butylhydroxytoluene (BHT); tert-butylhydroquinone (TBHQ); ascorbyl palmitate; rc-propyl gallate; salts thereof; and mixtures thereof. Suitable antioxidants and formulations containing such antioxidants are described in International Publication No. WO 2011/066651, which is hereby incorporated by reference.

In another embodiment, the lipid particles or compositions contain the antioxidant EDTA (or a salt thereof), the antioxidant citrate (or a salt thereof), or EDTA (or a salt thereof) in combination with one or more (e.g., a mixture of) primary and/or secondary antioxidants such as α-tocopherol (or a salt thereof) and/or ascorbyl palmitate (or a salt thereof).

In one embodiment, the antioxidant is present in an amount sufficient to prevent, inhibit, or reduce the degradation of the cationic lipid present in the lipid particle. For example, the antioxidant may be present at a concentration of at least about or about 0.1 mM, 0.5 mM, 1 mM, 10 mM, 100 mM, 500 mM, 1 M, 2 M, or 5M, or from about 0.1 mM to about 1 M, from about 0.1 mM to about 500 mM, from about 0.1 mM to about 250 mM, or from about 0.1 mM to about 100 mM.

The lipid particles and compositions described herein can further include an apolipoprotein. As used herein, the term "apolipoprotein" or "lipoprotein" refers to apolipoproteins known to those of skill in the art and variants and fragments thereof and to apolipoprotein agonists, analogues or fragments thereof described below.

In a preferred embodiment, the active agent is a nucleic acid, such as a siRNA. For example, the active agent can be a nucleic acid encoded with a product of interest, including but not limited to, RNA, antisense oligonucleotide, an antagomir, a DNA, a plasmid, a ribosomal RNA (rRNA), a micro RNA (miRNA) (e.g., a miRNA which is single stranded and 17-25 nucleotides in length), transfer RNA (tRNA), a small interfering RNA (siRNA), small nuclear RNA (snRNA), antigens, fragments thereof, proteins, peptides, vaccines and small molecules or mixtures thereof. In one more preferred embodiment, the nucleic acid is an oligonucleotide (e.g., 15-50 nucleotides in length (or 15-30 or 20-30 nucleotides in length)). An siRNA can have, for instance, a duplex region that is 16-30 nucleotides long. In another embodiment, the nucleic acid is an immunostimulatory oligonucleotide, decoy oligonucleotide, supermir, miRNA mimic, or miRNA inhibitor. A supermir refers to a single stranded, double stranded or partially double stranded oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or both or modifications thereof, which has a nucleotide sequence that is substantially identical to an miRNA and that is antisense with respect to its target. miRNA mimics represent a class of molecules that can be used to imitate the gene silencing ability of one or more miRNAs. Thus, the term "microRNA mimic" refers to synthetic non-coding RNAs (i.e. the miRNA is not obtained by purification from a source of the endogenous miRNA) that are capable of entering the RNAi pathway and regulating gene expression.

The nucleic acid that is present in a lipid-nucleic acid particle can be in any form. The nucleic acid can, for example, be single-stranded DNA or RNA, or double-stranded DNA or RNA, or DNA-RNA hybrids. Non-limiting examples of double-stranded RNA include siRNA. Single-stranded nucleic acids include, *e.g.*, antisense oligonucleotides, ribozymes, microRNA, and triplex-forming oligonucleotides. The lipid particles of the present invention can also deliver nucleic acids which are conjugated to one or more ligands.

Pharmaceutical Compositions

The lipid particles, particularly when associated with a therapeutic agent, may be formulated as a pharmaceutical composition, e.g., which further comprises a pharmaceutically acceptable diluent, excipient, or carrier, such as physiological saline or phosphate buffer.

The resulting pharmaceutical preparations may be sterilized by conventional, well known sterilization techniques. The aqueous solutions can then be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, and tonicity adjusting agents, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, and calcium chloride. Additionally, the lipidic suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as α-tocopherol and water-soluble iron-specific chelators, such as ferrioxamine, are suitable.

The concentration of lipid particle or lipid-nucleic acid particle in the pharmaceutical formulations can vary, for example, from less than about 0.01%, to at or at least about 0.05-5% to as much as 10 to 30% by weight.

Methods of Manufacture

Methods of making cationic lipids, lipid particles containing them, and pharmaceutical compositions containing the cationic lipids and/or lipid particles are described in, for example, International Publication Nos. WO 2010/054406, WO 2010/054401, WO 2010/054405, WO 2010/054384, WO 2010/042877, WO 2010/129709, WO 2009/086558, and WO 2008/042973, and U.S. Patent Publication Nos. 2004/0142025, 2006/0051405 and 2007/0042031, each of which is incorporated by reference in its entirety.

For example, in one embodiment, a solution of one or more lipids (including a cationic lipid of any of the embodiments described herein) in an organic solution (e.g., ethanol) is prepared. Similarly, a solution of one or more active (therapeutic) agents (such as, for example an siRNA molecule or a 1:1 molar mixture of two siRNA molecules) in an aqueous buffered (e.g., citrate buffer) solution is prepared. The two solutions are mixed and diluted to form a colloidal suspension of siRNA lipid particles. In one embodiment, the siRNA lipid particles have an average particle size of about 80-90 nm. In further embodiments, the dispersion may be filtered through 0.45/2 micron filters, concentrated and diafiltered by tangential flow filtration.

Definitions

As used herein, the term "cationic lipid" inlcudes those lipids having one or two fatty acid or fatty aliphatic chains and an amino acid containing head group that may be protonated to form a cationic lipid at physiological pH. In some embodiments, a cationic lipid is referred to as an "amino acid conjugate cationic lipid."

A subject or patient in whom administration of the complex is an effective therapeutic regimen for a disease or disorder is preferably a human, but can be any animal, including a laboratory animal in the context of a clinical trial or screening or activity experiment. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods, compounds and compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, humans, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, and cats, avian species, such as chickens, turkeys, and songbirds, i.e., for veterinary medical use.

Many of the chemical groups recited in the generic formulas above are written in a particular order (for example, -OC(O)-). It is intended that the chemical group is to be incorporated into the generic formula in the order presented unless indicated otherwise. For example, a generic formula of the form $–(R)_i-(M^1)_k-(R)_m-$ where M^1 is –C(O)O- and k is 1 refers to $–(R)_i-C(O)O-(R)_m-$ unless specified otherwise. It is to be understood that when a chemical group is written in a particular order, the reverse order is also contemplated unless otherwise specified. For example, in a generic formula $–(R)_i-(M^1)_k-(R)_m-$ where M^1 is defined as –C(O)NH- (i.e., $–(R)_i-C(O)-NH-(R)_m-$), the compound where M^1 is –NHC(O)- (i.e., $–(R)_i-NHC(O)-(R)_m-$) is also contemplated unless otherwise specified.

The term “biodegradable cationic lipid” refers to a cationic lipid having one or more biodegradable groups located in the mid- or distal section of a lipidic moiety (e.g., a hydrophobic chain) of the cationic lipid. The incorporation of the biodegradable group(s) into the cationic lipid results in faster metabolism and removal of the cationic lipid from the body following delivery of the active pharmaceutical ingredient to a target area.

As used herein, the term “biodegradable group” refers to a group that include one or more bonds that may undergo bond breaking reactions in a biological environment, e.g., in an organism, organ, tissue, cell, or organelle. For example, the biodegradable group may be metabolizable by the body of a mammal, such as a human (e.g., by hydrolysis). Some groups that contain a biodegradable bond include, for example, but are not limited to esters, dithiols, and oximes. Non-limiting examples of biodegradable groups are -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, or -OC(O)(CR^3R^4)C(O)-.

As used herein, an “aliphatic” group is a non-aromatic group in which carbon atoms are linked into chains, and is either saturated or unsaturated.

The terms "alkyl" and “alkylene” refer to a straight or branched chain saturated hydrocarbon moiety. In one embodiment, the alkyl group is a straight chain saturated hydrocarbon. Unless otherwise specified, the “alkyl” or “alkylene” group contains from 1 to 24 carbon atoms. Representative saturated straight chain alkyl groups include methyl, ethyl, n-propyl, n-butyl, n-pentyl, and n-hexyl. Representative saturated branched alkyl groups include isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, and isopentyl.

The term "alkenyl" refers to a straight or branched chain hydrocarbon moiety having one or more carbon-carbon double bonds. In one embodiment, the alkenyl group contains 1, 2, or 3 double bonds and is otherwise saturated. Unless otherwise specified, the “alkenyl” group contains from 2 to 24 carbon atoms. Alkenyl groups include both cis and trans isomers. Representative straight chain and branched alkenyl groups include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, and 2,3-dimethyl-2-butenyl.

The term "alkynyl" refers to a straight or branched chain hydrocarbon moiety having one or more carbon-carbon triple bonds. Unless otherwise specified, the “alkynyl” group contains from 2 to 24 carbon atoms. Representative straight chain and branched alkynyl groups include acetylenyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, and 3-methyl-1-butynyl.

Unless otherwise specified, the terms “branched alkyl”, “branched alkenyl”, and “branched alkynyl” refer to an alkyl, alkenyl, or alkynyl group in which one carbon atom in the group (1) is bound to at least three other carbon atoms and (2) is not a ring atom of a cyclic group. For example, a spirocyclic group in an alkyl, alkenyl, or alkynyl group is not considered a point of branching.

Unless otherwise specified, the term "acyl" refers to a carbonyl group substituted with hydrogen, alkyl, partially saturated or fully saturated cycloalkyl, partially saturated or fully saturated heterocycle, aryl, or heteroaryl. For example, acyl groups include groups such as (C_1-C_{20})alkanoyl (e.g., formyl, acetyl, propionyl, butyryl, valeryl, caproyl, and t-butylacetyl), (C_3-C_{20})cycloalkylcarbonyl (e.g., cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl, and cyclohexylcarbonyl), heterocyclic carbonyl (e.g., pyrrolidinylcarbonyl, pyrrolid-2-one-5-carbonyl, piperidinylcarbonyl, piperazinylcarbonyl, and tetrahydrofuranylcarbonyl), aroyl (e.g., benzoyl) and heteroaroyl (e.g., thiophenyl-2-carbonyl, thiophenyl-3-carbonyl, furanyl-2-carbonyl, furanyl-3-carbonyl, lH-pyrroyl-2-carbonyl, 1H-pyrroyl-3-carbonyl, and benzo[b]thiophenyl-2-carbonyl).

The term "aryl" refers to an aromatic monocyclic, bicyclic, or tricyclic hydrocarbon ring system. Unless otherwise specified, the “aryl” group contains from 6 to 14 carbon atoms. Examples of aryl moieties include, but are not limited to, phenyl, naphthyl, anthracenyl, and pyrenyl.

The terms “cycloalkyl” and “cycloalkylene” refer to a saturated monocyclic or bicyclic hydrocarbon moiety such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. Unless otherwise specified, the “cycloalkyl” or “cycloalkylene” group contains from 3 to 10 carbon atoms.

The term “cycloalkylalkyl” refers to a cycloalkyl group bound to an alkyl group, where the alkyl group is bound to the rest of the molecule.

The term "heterocycle" (or “heterocyclyl”) refers to a non-aromatic 5- to 8-membered monocyclic, or 7- to 12-membered bicyclic, or 11- to 14-membered tricyclic ring system which is either saturated or unsaturated, and which contains from 1 to 3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized. For instance, the heterocycle may be a cycloalkoxy group. The heterocycle may be attached to the rest of the molecule via any heteroatom or carbon atom in the heterocycle. Heterocycles include, but are not limited to, morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizynyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, and tetrahydrothiopyranyl.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 7-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, where the heteroatoms are selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). The heteroaryl groups herein described may also contain fused rings that share a common carbon-carbon bond.

The term "substituted", unless otherwise indicated, refers to the replacement of one or more hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: halo, alkyl, alkenyl, alkynyl, aryl, heterocyclyl, thiol, alkylthio, oxo, thioxy, arylthio, alkylthioalkyl, arylthioalkyl, alkylsulfonyl, alkylsulfonylalkyl, arylsulfonylalkyl, alkoxy, aryloxy, aralkoxy, aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl, alkoxycarbonyl, aryloxycarbonyl, haloalkyl, amino, trifluoromethyl, cyano, nitro, alkylamino, arylamino, alkylaminoalkyl, arylaminoalkyl, aminoalkylamino, hydroxy, alkoxyalkyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonylalkyl, acyl, aralkoxycarbonyl, carboxylic acid, sulfonic acid, sulfonyl, phosphonic acid, aryl, heteroaryl, heterocyclic, and an aliphatic group. It is understood that the substituent may be further substituted. Exemplary substituents include amino, alkylamino, dialkylamino, and cyclic amino compounds.

The term "halogen" or "halo" refers to fluoro, chloro, bromo and iodo.

The following abbreviations may be used in this application:

DSPC: distearoylphosphatidylcholine; DPPC: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine; POPC: 1- palmitoyl-2-oleoyl-sn-phosphatidylcholine; DOPE: 1,2-dileoyl-sn-3-phosphoethanolamine; PEG-DMG generally refers to 1,2-dimyristoyl-sn-glycerol-methoxy polyethylene glycol (e.g., PEG 2000); TBDPSCl: tert-Butylchlorodiphenylsilane; DMAP: dimethylaminopyridine; HMPA: hexamethylphosphoramide; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; DIPEA: diisopropylethylamine; DCM: dichloromethane; TEA: triethylamine; TBAF: tetrabutylammonium fluoride

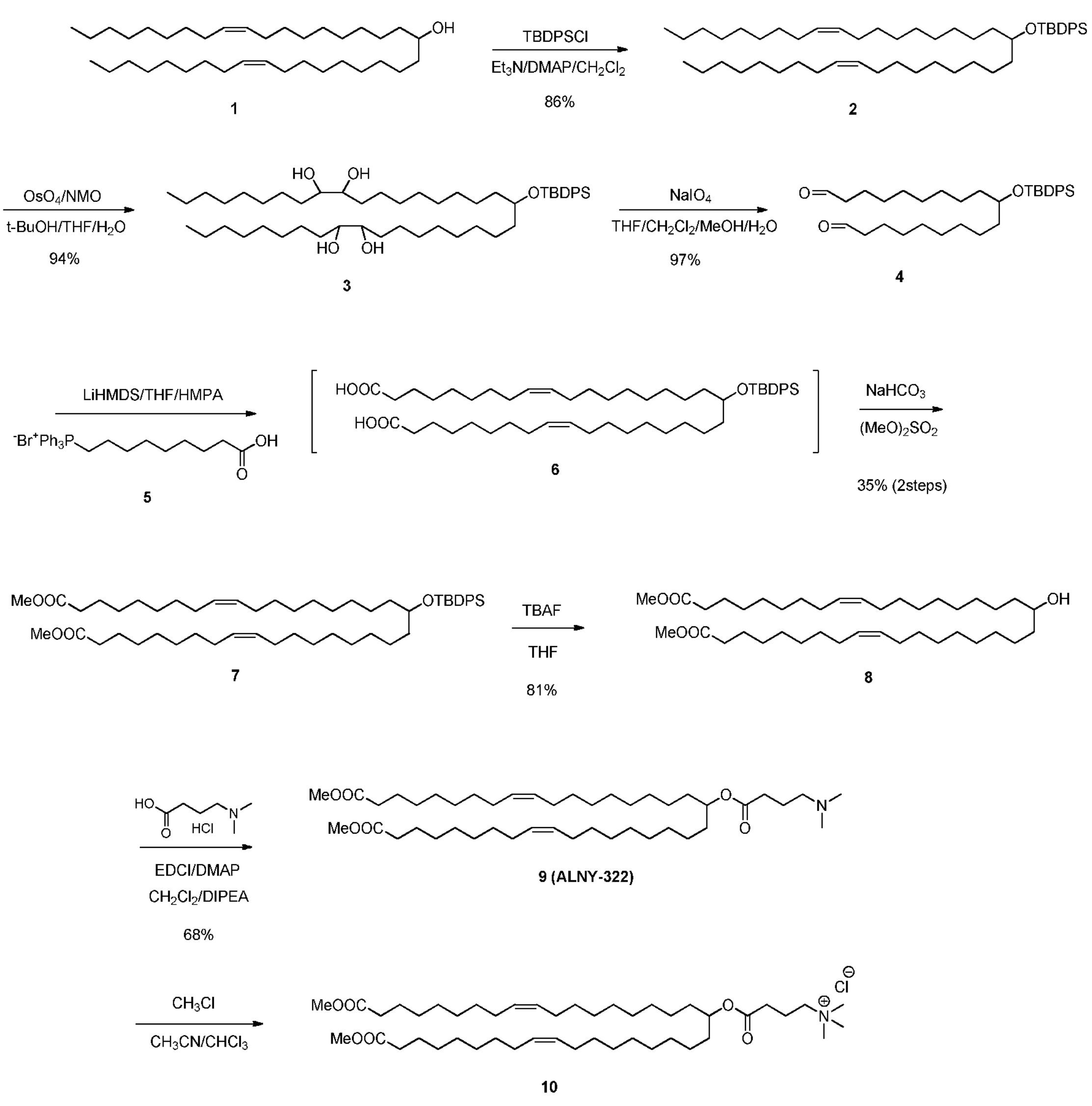
Methods to prepare various organic groups and protective groups are known in the art and their use and modification is generally within the ability of one of skill in the art (*see, for example*, Green, T.W. *et. al.,* Protective Groups in Organic Synthesis (1999); Stanley R. Sandler and Wolf Karo, Organic Functional Group Preparations (1989); Greg T. Hermanson, Bioconjugate Techniques (1996); and Leroy G. Wade, Compendium Of Organic Synthetic Methods (1980)). Briefly, protecting groups are any group that reduces or eliminates unwanted reactivity of a functional group. A protecting group can be added to a functional group to mask its reactivity during certain reactions and then removed to reveal the original functional group. In some embodiments an "alcohol protecting group" is used. An "alcohol protecting group" is any group which decreases or eliminates unwanted reactivity of an alcohol functional group. Protecting groups can be added and removed using techniques well known in the art.

The compounds may be prepared by at least one of the techniques described herein or known organic synthesis techniques.

Examples

Example 1:

Scheme 1. ALNY-322/quaternary salts



Compound **2**: To a solution of compound **1** (10.0 g, 18.8 mmol, *see* International Publication No. WO 2010/054406) in CH_2Cl_2 (80 mL) were added triethylamine (7.86 mL, 56.4 mmol), DMAP (459 mg, 3.76 mmol) and *tert*-butyl(chloro)diphenylsilane (9.62 mL, 37.6 mmol). The reaction mixture was stirred for 24 hours. The mixture was then diluted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$ solution. The organic layer was separated and dried over anhydrous

Na_2SO_4. After filtration and concentration, the crude product was purified by silica gel column chromatography (0-5% EtOAc in hexane) to afford **2** (12.4 g, 16.1 mmol, 86%, R_f = 0.24 with hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 7.66-7.68 (m, 4 H), 7.33-7.42 (m, 6 H), 5.30-5.39 (m, 4 H), 3.67-3.72 (m, 1 H), 1.97-2.04 (m, 8 H), 1.07-1.42 (m, 52 H), 1.05 (s, 9 H), 0.88 (t, *J* = 6.8 Hz, 6 H).

Compound **3**: To a solution of **2** (12.4 g, 16.1 mmol) in *tert*-butanol (100 mL), THF (30 mL) and H_2O (10 mL) were added 4-methylmorpholine *N*-oxide (4.15 g, 35.4 mmol) and osmium tetroxide (41 mg, 0.161 mg). The reaction mixture was stirred for 16 hours, then quenched by adding sodium bisulfite. After removing the solvents by evaporation, the residue was extracted with Et_2O (500 mL) and H_2O (300 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude was purified by silica gel column chromatography (hexane:EtOAc = 1:1, R_f = 0.49) to afford **3** (12.7 g, 15.1 mmol, 94%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.66-7.68 (m, 4 H), 7.33-7.43 (m, 6 H), 3.67-3.73 (m, 1 H), 3.57-3.62 (m, 4 H), 1.82 (t, *J* = 5.0 Hz, 4 H), 1.10-1.51 (m, 60 H), 1.04 (s, 9 H), 0.88 (t, *J* = 6.8 Hz, 6 H).

Compound **4**: To a solution of **3** (12.6 g, 15.0 mmol) in 1,4-dioxane (220 mL), CH_2Cl_2 (70 mL), MeOH (55 mL), and H_2O (55 mL) was added $NaIO_4$ (7.70 g, 36.0 mmol). The reaction mixture was stirred for 16 hours at room temperature. The mixture was extracted with Et_2O (500 mL) and H_2O (300 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by silica gel column chromatography (Hexane:EtOAc = 9:1, R_f = 0.30) to afford **4** (7.98 g, 14.5 mmol, 97%). Molecular weight for $C_{35}H_{54}NaO_3Si$ $(M+Na)^+$ Calc. 573.3740, Found 573.3.

Compound **7**: To a solution of **5** (*see*, Tetrahedron, 63, 1140-1145, 2006; 1.09 g, 2.18 mmol) in THF (20 mL) and HMPA (4 mL), LiHMDS (1 M THF solution, 4.36 mL, 4.36 mmol) was added at -20 °C. The resulting mixture was stirred for 20 minutes at the same temperature, then cooled to -78 °C. A solution of **4** (500 mg, 0.908 mmol) in THF (4 mL) was added. The mixture was stirred and allowed to warm to room temperature overnight. MS analysis showed the formation of the di-acid (**6**; $C_{53}H_{85}O_5Si$ $(M-H)^-$ calc. 829.6166, observed 829.5). To the mixture, $NaHCO_3$ (1.10 g, 13.1 mmol) and dimethyl sulfate (1.24 mL, 13.1 mmol) were added and stirred for 2 hours at room temperature. The reaction was quenched by adding saturated NH_4Cl aqueous solution (50 mL) then extracted with Et_2O (2 x 100 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by silica gel column chromatography (Hexane:EtOAc = 9:1, R_f = 0.35) to afford **7** (270 mg, 0.314 mmol, 35%). Molecular weight for $C_{55}H_{90}NaO_5Si$ $(M+Na)^+$ Calc. 881.6455, Found 881.6484.

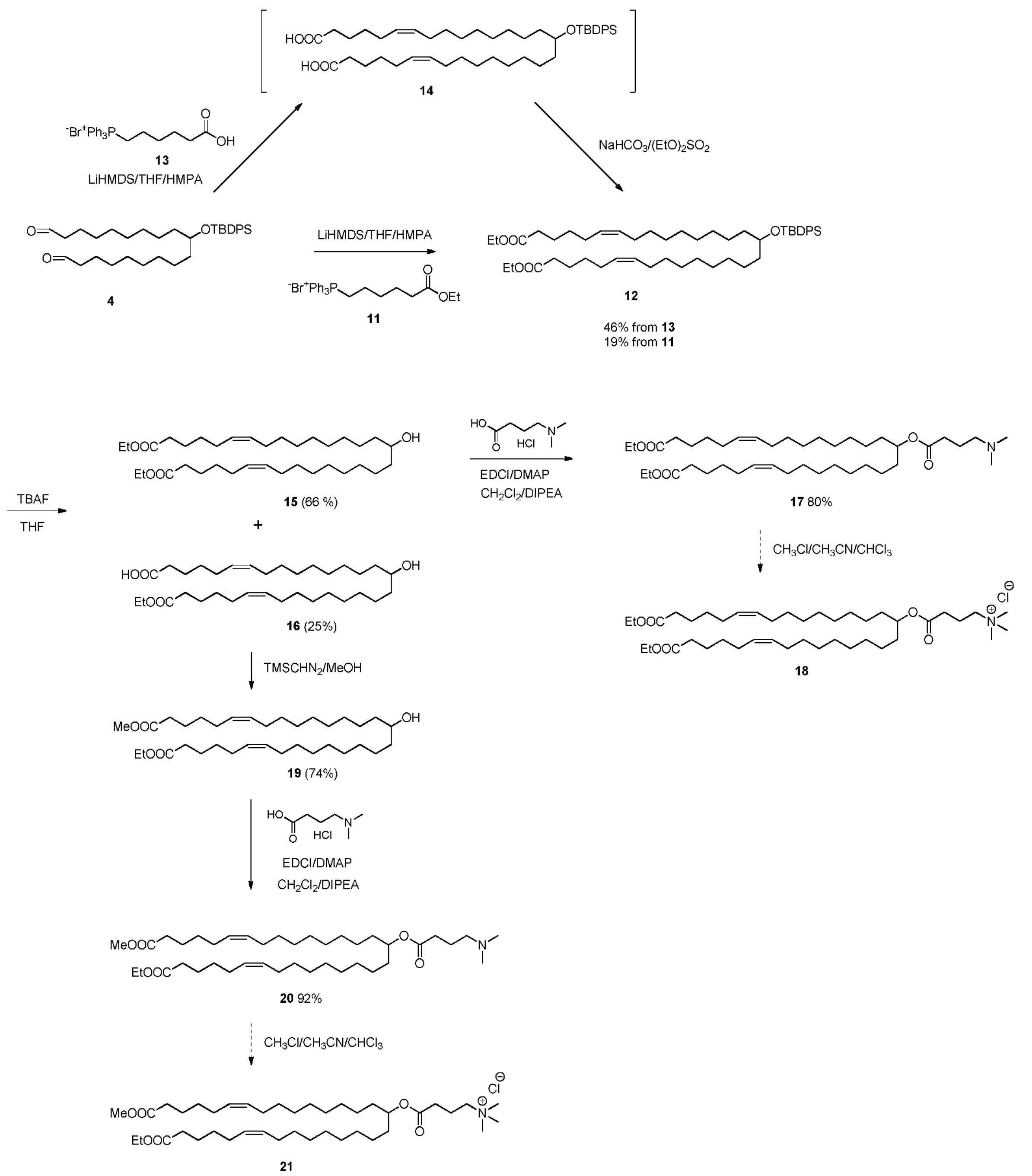
Compound **8**: To a solution of **7** (265 mg, 0.308 mmol) in THF (2.5 mL), *n*-TBAF (1 M THF solution, 0.555 mL, 0.555 mmol) was added. The reaction mixture was stirred for 14 hours at 45 ^oC. After concentration, the mixture was purified by silica gel column chromatography (Hexane:EtOAc = 3:1, R_f = 0.52) to afford **8** (155 mg, 0.250 mmol, 81%). Molecular weight for $C_{39}H_{72}NaO_5$ $(M+Na)^+$ Calc. 643.5277, Found 643.5273.

Compound **9**: To a solution of compound **8** (150 mg, 0.242 mmol) and 4-(dimethylamino)butyric acid hydrochloride (49 mg, 0.290 mmol) in CH_2Cl_2 (5 mL) were added diisopropylethylamine (0.126 mL, 0.726 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (56 mg, 0.290 mmol) and DMAP (6 mg, 0.0484 mmol). The reaction mixture was stirred at room temperature for 14 hours. The reaction mixture was then diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ aq. (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2) to afford compound **9** (121 mg, 0.165 mmol, 68%, R_f = 0.25 developed with 5% MeOH in CH_2Cl_2). Molecular weight for $C_{45}H_{84}NO_6$ $(M+H)^+$ Calc. 734.6299, Found 734.5.

Compound **10**: Treatment of compound **9** with CH_3Cl in CH_3CN and $CHCl_3$ can afford compound **10**.

Example 2:

Scheme 2



Compound **12**: To a solution of **11** (Journal of Medicinal Chemistry (1995), 38, 636-46; 1.25 g, 2.58 mmol) in THF (20 mL) and HMPA (4 mL), LiHMDS (1 M THF solution, 2.58 mL, 2.58

mmol) was added at -20 °C. The mixture was stirred for 20 min at the same temperature, then cooled to -78 °C. A solution of **4** (500 mg, 0.908 mmol) in THF (9 mL) and HMPA (0.9 mL) was added. The mixture was stirred from -78 °C to room temperature overnight. The reaction was quenched by adding H_2O (40 mL) then extracted with Et_2O (150 mL x 3). The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude was purified by silica gel column chromatography (Hexane:EtOAc = 9:1, R_f = 0.35) to give **12** (136 mg, 0.169 mmol, 19%). Molecular weight for $C_{51}H_{82}NaO_5Si$ $(M+Na)^+$ Calc. 825.5829, Found 825.5.

Using **13** in place of **5**, a procedure analogous to that described for compound **7** was followed to afford compound **12** (135 mg, 0.168 mmol, 46%).

Compound **15**/Compound **16**: To a solution of **12** (800 mg, 0.996 mmol) in THF (5 mL), *n*-TBAF (1 M THF solution, 5 mL, 5.00 mmol) was added. The reaction mixture was stirred for 16 h at 45 °C. After concentration, the mixture was purified by silica gel column chromatography to give **15** (Hexane:EtOAc = 3:1, R_f = 0.46, 372 mg, 0.659 mmol, 66%) and **16** (CH_2Cl_2:MeOH = 95:5, R_f = 0.36, 135 mg, 0.251 mmol, 25%). Molecular weight for **15**; $C_{35}H_{64}NaO_5$ $(M+Na)^+$ Calc. 587.4651, Found 587.4652. Molecular weight for **16**; $C_{33}H_{61}O_5$ $(M+H)^+$ Calc. 537.4519, Found 537.5.

Compound **17**: To a solution of compound **15** (164 mg, 0.290 mmol) and 4-(dimethylamino)butyric acid hydrochloride (58 mg, 0.348 mmol) in CH_2Cl_2 (5 mL) were added diisopropylethylamine (0.152 mL, 0.870 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (67 mg, 0.348 mmol) and DMAP (7 mg, 0.058 mmol). The reaction mixture was stirred at room temperature for 14 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ aq. (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2) to give compound **17** (158 mg, 0.233 mmol, 80%, R_f = 0.24 developed with 5% MeOH in CH_2Cl_2). Molecular weight for $C_{45}H_{84}NO_6$ $(M+H)^+$ Calc. 734.6299, Found 734.5.

Compound **18**: Treatment of compound **17** with CH_3Cl in CH_3CN and $CHCl_3$ can afford compound **18**.

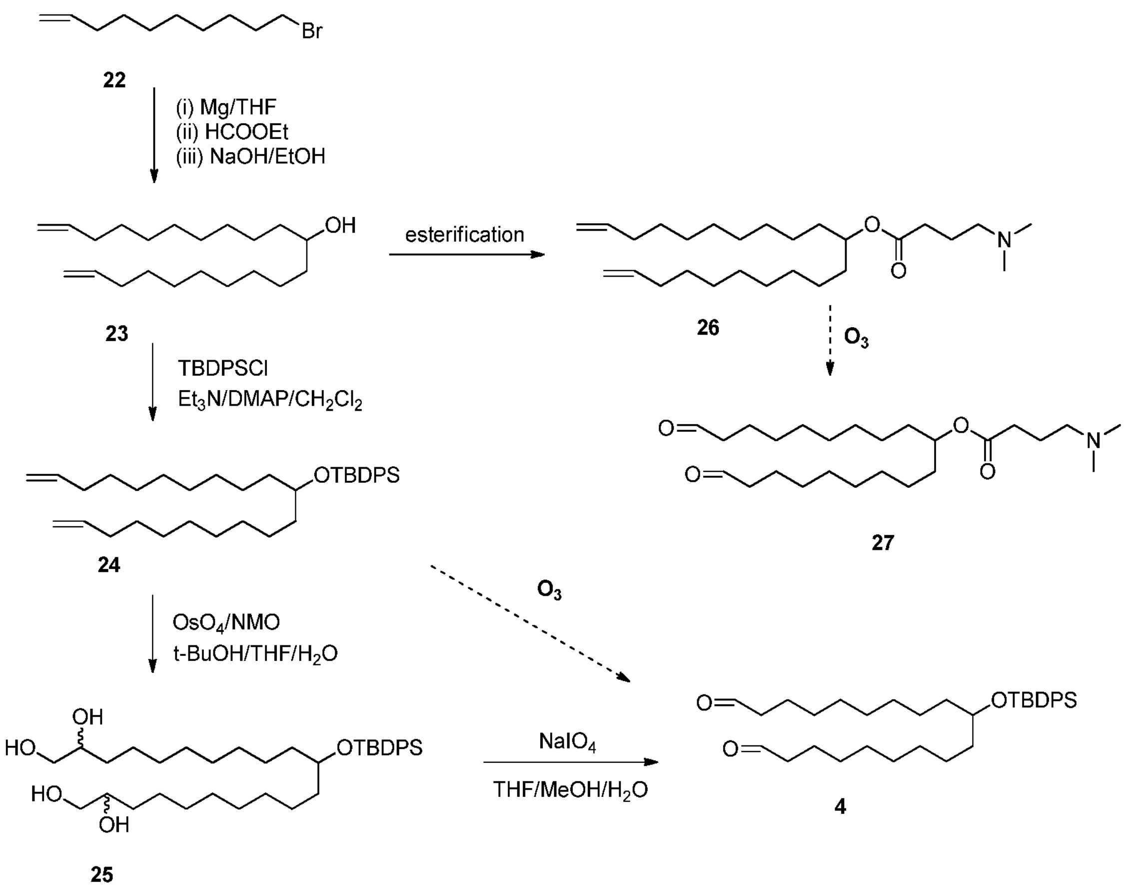
Compound **19**: To a solution of **16** (130 mg, 0.242 mmol) in THF (2 mL) and MeOH (2 mL), trimethylsilyldiazomethane (2 M solution in Et_2O, 0.158 mL, 0.315 mmol) was added. The reaction mixture was stirred for 14 h. After evaporation, the residue was purified by silica gel column chromatography (Hexane:EtOAc = 3:1, $R_f = 0.50$) to give **19** (99 mg, 0.180 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29-5.40 (m, 4 H), 4.12 (q, *J* = 7.1 Hz, 2 H), 3.66 (s, 3 H), 3.55-3.59 (m, 1 H), 2.30 (dd, *J* = 14.7, 7.2 Hz, 4 H), 1.98-2.07 (m, 8 H), 1.60-1.68 (m, 4 H), 1.23-1.43 (m, 37 H).

Compound **20**: To a solution of compound **19** (95 mg, 0.168 mmol) and 4-(dimethylamino)butyric acid hydrochloride (42 mg, 0.252 mmol) in CH_2Cl_2 (3 mL) were added diisopropylethylamine (0.088 mL, 0.504 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (48 mg, 0.504 mmol) and DMAP (4 mg, 0.034 mmol). The reaction mixture was stirred at room temperature for 14 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ aq. (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2) to give compound **20** (103 mg, 0.155 mmol, 92%, R_f = 0.19 developed with 5% MeOH in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29-5.40 (m, 4 H), 4.83-4.89 (m, 1 H), 4.12 (q, *J* = 7.1 Hz, 2 H), 3.67 (s, 3 H), 2.28-2.34 (m, 8 H), 2.23 (s, 6 H), 1.98-2.07 (m, 8 H), 1.76-1.83 (m, 2 H), 1.60-1.68 (m, 4 H), 1.23-1.51 (m, 35 H).

Compound **21**: Treatment of compound **20** with CH_3Cl in CH_3CN and $CHCl_3$ can afford compound **21**.

Example 3: **Alternate Synthesis for Di-Aldehyde Intermediate 4**

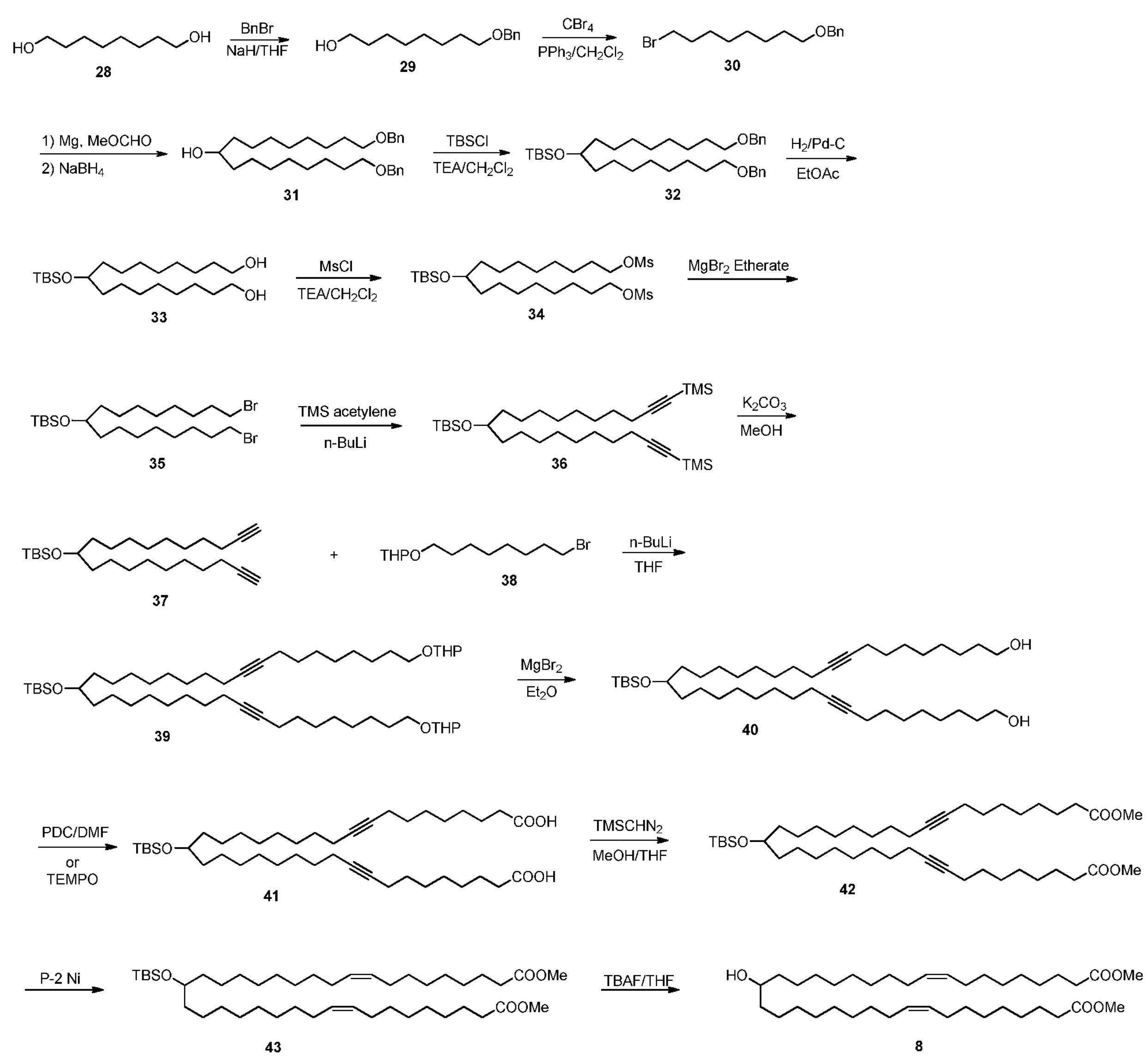
Scheme 3



The di-aldehyde **4** can be synthesized as shown in Scheme 3, using 1-bromo-9-decene. Di-aldehyde containing a head group **27** can be useful for the synthesis of terminal ester-substituted lipids using, e.g., a Wittig reaction. Ozonolysis can afford di-aldehyde **4** and **27**.

Example 4: Alternate Synthesis for Compound 8

Scheme 4



Compound **8** can be synthesized as shown in **Scheme 4**.

Compound **29**: To a stirred suspension of NaH (60% in oil, 82 g, 1.7096 mol) in 500mL anhydrous DMF, a solution of compound **28** (250 g, 1.7096 mol) in 1.5 L DMF was added slowly using a dropping funnel at 0 °C. The reaction mixture was stirred for 30 minutes, then benzyl bromide (208.86 mL, 1.7096 mol) was added slowly under an atmosphere of nitrogen. The reaction was then warmed to ambient temperature and stirred for 10 hours. The mixture was then quenched with crushed ice (~2 kg) and extracted with ethyl acetate (2 x 1 L). The organic layer was washed with water (1L) to remove unwanted DMF, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The crude compound was purified on 60-120 silica gel, eluted with 0-5% MeOH in DCM to afford compound **29** (220 g, 54%) as a pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33-7.24 (m, 5 H), 4.49 (s, 2 H), 3.63-3.60 (m, 2 H), 3.47-3.43 (m, 2 H), 1.63-1.51 (m, 4 H), 1.39-1.23 (m, 8 H).

Compound **30**: Compound **29** (133 g, 0.5635 mol) was dissolved in 1.5 L of DCM, CBr_4 (280.35 g, 0.8456 mol) was added into this stirring solution and the reaction mixture was cooled to 0°C under an inert atmosphere. PPh_3 (251.03 g, 0.9571 mol) was then added in portions keeping the temperature below 20 °C. After complete addition, the reaction mixture was stirred for 3 hours at room temperature. After completion of the reaction, the solid (PPh_3O) that precipitated from the reaction mixture was removed by filtration, and the filtrate was diluted with crushed ice (~ 1.5 kg) and extracted with DCM (3 x 750 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and distilled under vacuum. The resulting crude compound was chromatographed on 60-120 mesh silica gel column using 0-5 % ethyl acetate in hexanes as eluting system to afford compound **30** (150 g, 89%) as pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33-7.25 (m, 5 H), 4.49 (s, 2 H), 3.47-3.41 (m, 2 H), 3.41-3.37 (m, 2 H), 1.86-1.80 (m, 4 H), 1.62-1.56 (m, 2 H), 1.42-1.29 (m, 8 H).

Compound **31**: To freshly activated Mg turnings (24.08 g, 1.003 mol) was added 200 mL anhydrous THF, followed by the addition of pinch of iodine into the mixture under an inert atmosphere. A solution of Compound **30** (150 g, 0.5016 mol) in 1 L of dry THF was added slowly, controlling the exothermic reaction. The reaction was then heated to reflux for 1 hour, then cooled to room temperature. Methyl formate (60.24 g, 1.0033 mol) was then added slowly and the reaction was continued for 2 hours. After completion, the reaction was quenched by slow addition of 10% HCl followed by water (1 L) and extracted with ethyl acetate (3 x 1 L). The organic layer was taken in 5 litre beaker, diluted with 500 mL of methanol and cooled to 0 °C. To this solution, an excess of $NaBH_4$ (~ 5eq) was added in portions to ensure hydrolysis of the formate ester which was not cleaved by addition of HCl. The resulting solution was stirred for an hour and then volatilites were removed under vacuum. The residue was taken in water (1 L) and acidified by 10% HCl solution (pH 4). The product was then extracted with ethyl acetate (3 x 1

L). the organic phase was then dried and concentrated on rotary evaporator to afford the desired compound **31** (57 g, 24%) as solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35-7.32 (m, 8 H), 7.29-7.24 (m, 2 H), 4.49 (s, 4 H), 3.56 (m, 1 H), 3.46-3.43 (m, 4 H), 1.63-1.56 (m, 4 H), 1.44-1.34 (m, 28 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.56, 128.21, 127.49, 127.34, 72.72, 71.76, 70.37, 37.37, 29.64, 29.56, 29.47, 29.33, 26.07, 25.54.

Compound **32**: Compound **31** (56 g, 0.1196 mol) was dissolved in 700 mL dry THF and cooled to 0 °C. TBSCl (36.06 g, 0.2396 mol) was added slowly followed by the addition of imidazole (32.55 g, 0.4786 mol) under an inert atmosphere. The reaction was then stirred at room temperature for 18 hours. Upon completion, the reaction was quenched with ice (~1 kg) and extracted with ethyl acetate (3 x 500 mL). The organic layer was separated, washed with saturated $NaHCO_3$ solution to remove acidic impurities, dried over Na_2SO_4 and evaporated under reduce pressure to afford a crude compound that was purified by silica gel (60-120 mesh) and eluted with 0- 10% ethyl acetate hexane to afford (60 g, 82%) of compound **32** as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33-7.24 (m, 10 H), 4.49 (s, 4 H), 3.60-3.57 (m, 1 H), 3.46-3.43 (m, 4 H), 1.61-1.54 (m, 4 H), 1.41-1.26 (m, 28 H), 0.87 (s, 9 H), 0.02 (s, 6 H).

Compound **33**: Compound **32** (60 g, 0.1030 mol) was dissolved in 500 mL ethyl acetate and degassed with N_2 for 20 minutes. (10 wt %) Pd on carbon (12 g) was added and the reaction was stirred under an atmosphere of hydrogen for 18 hours. After completion, the mixture was filtered through a bed of celite and washed with ethyl acetate. The filtrate was evaporated under vacuum to afford compound **33** (19 g, 46%) that was pure enough to use in the next synthetic sequence. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.64-3.58 (m, 5 H), 1.59 (br, 2 H), 1.57-1.51 (m, 4 H), 1.38-1.22 (m, 28 H), 0.87 (s, 9 H), 0.02 (s, 6 H).

Compound **34**: Compound **33** (8.2 g, 0.0199 mol) was dissolved in 100 mL dry DCM and cooled to 0 °C. TEA (22.14 mL, 0.1592 mol) was added under an inert atmosphere. After stirring the mixture for 5 minutes, mesyl chloride (4.6 mL, 0.059 mol) was added drop wise and the reaction was stirred further for 3 hours. After completion of the reaction, the mixture was quenched with ice (~200 g) and extracted with DCM (3 x 75 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford a crude compound which was purified on a 60-120 mesh silica gel column using 0-30% ethyl acetate in hexane as eluting system to afford compound **34** (8.2 g, 73%) as a pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.22-4.19 (m, 4 H), 3.60-3.58 (m, 1 H), 2.99 (s, 6 H), 1.75-1.69 (m, 4 H), 1.38-1.28 (m, 28 H), 0.86 (s, 9 H), 0.02 (s, 6 H).

Compound **35:** To a solution of compound **34** (8.2 g, 0.0146 mol) in 400 mL dry ether was added $MgBr_2 \cdot Et_2O$ (22.74 g, 0.08817 mol) in portions at 0 °C under a nitrogen atmosphere. After complete addition, the reaction mixture was heated to reflux for 28 hours. After completion of reaction, inorganic material formed in the reaction was removed by filtration. The filtrate was evaporated and the resulting crude compound was purified on 60-120 mesh silica gel column using 0-3% ethyl acetate in hexanes as eluting system to afford compound **35** (6.6 g, 85%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.61-3.58 (m, 1 H), 3.41-3.37 (t, 4 H, *J* = 6.8 Hz), 1.87-1.80 (m, 4 H), 1.42-1.25 (m, 24 H), 0.87 (s, 9 H), 0.012 (s, 6 H).

Compound **36**: A solution of ethynyl trimethyl silane (5.3 mL, 0.0378 mol) in 60 mL dry THF was cooled to -78°C and 1.4 M *n*-BuLi (23 mL, 0.03405 mol) in hexane was added slowly under an inert atmosphere. The reaction was stirred for 10 minutes, then HMPA (2.3 g, 0.01324 mol) was added and the resulting mixture was then stirred for 2 hours at 0 °C, then cooled to -78 °C. To this a solution of compound **35** (5 g, 0.0094 mol) in 60 mL dry THF was added slowly and after complete addition, the reaction was warmed to room temperature and maintained for 18 hours. The reaction progress was monitored by ^{1}H NMR. After completion, the reaction mixture was cooled to 0 °C and quenched by careful addition of saturated NH_4Cl solution (50 mL) followed by water (200 mL). The aqueous phase was extracted with hexane (3 x 250 mL). The organic layer was dried and solvent removed under vacuum to afford compound **36** (5 g, 94%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.62-3.56 (m, 1 H), 2.21-2.17 (m, 4 H), 1.49-1.47 (m, 4 H), 1.37-1.26 (m, 24 H), 0.87 (s, 9 H), 0.13 (s, 18 H), 0.021 (s, 6 H).

Compound **37**: To a stirred solution of compound **36** (5 g, 0.0088 mol) in 50 mL methanol, was added K_2CO_3 (6.1 g, 0.044 mol) in one portion, and the resulting mixture was stirred for 18 hours at ambient temperature. Volatilities were then removed on a rotary evaporator and the crude mixture was diluted with 100 mL water and extracted with hexane (3 x 100 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum to afford compound **37** (3.5

g, 97%) which was used which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.60-3.58 (m, 1 H), 2.19-2.14 (m, 4 H), 1.93-1.92 (m, 2 H), 1.54-1.49 (m, 4 H), 1.37-1.27 (m, 24 H), 0.87 (s, 9 H), 0.02 (s, 6 H).

Compound **39**: Compound **37** (2.5 g, 0.00598 mol) was dissolved in 25 mL dry THF and cooled to -40 °C. *n*-BuLi (1.4 M in hexane 12.9 mL, 0.01794 mol) was added slowly, followed, after a 10 minute interval, by slow addition of HMPA (25 mL). The resulting mixture was maintained for 30 minutes -40 °C under a nitrogen atmosphere. A solution of compound **38** (3.5 g, 1.01196 mol) in 25 mL dry THF was then added drop wise to the cooled reaction mixture. The resulting mixture was warmed to room temperature over 2 hours, then stirred at room temperature for 18 hours. The mixture was then quenched by adding saturated NH_4Cl solution (~50 mL) and the product was extracted with ethyl acetate (3 x 50 mL). The solvent was removed on a rotary evaporator and the resulting crude product was purified by (100-200 mesh) silica gel column using 0-3% ethyl acetate in dichloromethane as eluting system to afford compound **39** (0.9 g, 18%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.56-4.55 (m, 2 H), 3.87-3.83 (m, 2 H), 3.74-3.68 (m, 2 H), 3.59-3.57 (m, 1 H), 3.49-3.46 (m, 2 H), 3.39-3.33 (m, 2 H), 2.13-2.10 (m, 8 H), 1.87-1.75 (m, 2 H), 1.74-1.66 (m, 2 H), 1.57-1.42 (m, 20 H), 1.40-1.19 (m, 40 H), 0.87 (s, 9 H), 0.02 (s, 6 H).

Compound **40**: To a solution of compound **39** (504 mg, 0.598 mmol) in 10 mL dry ether was added $MgBr_2 \cdot Et_2O$ (926 mg, 3.59 mmol). The reaction mixture was stirred for 14 hours, then quenched by adding saturated $NaHCO_3$ aqueous solution. The product was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford compound **40** (307 mg, 0.455 mmol, 76%, R_f = 0.36 developed with hexane:EtOAc = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59-3.66 (m, 5 H), 2.14 (t, *J* = 6.6 Hz, 8 H), 1.21-1.59 (m, 52 H), 0.88 (s, 9 H), 0.03 (s, 6 H).

Compound **41**: To a stirred solution of **40** (180 mg, 0.267 mmol) in anhydrous DMF (5 mL) was added pyridinium dichromate (603 mg, 1.60 mmol). The reaction mixture was stirred for 48 hours. After dilution with water (20 mL), the mixture was extracted with Et_2O (3 x 40 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford compound **41** (53 mg, 0.075 mmol, 28%, R_f =

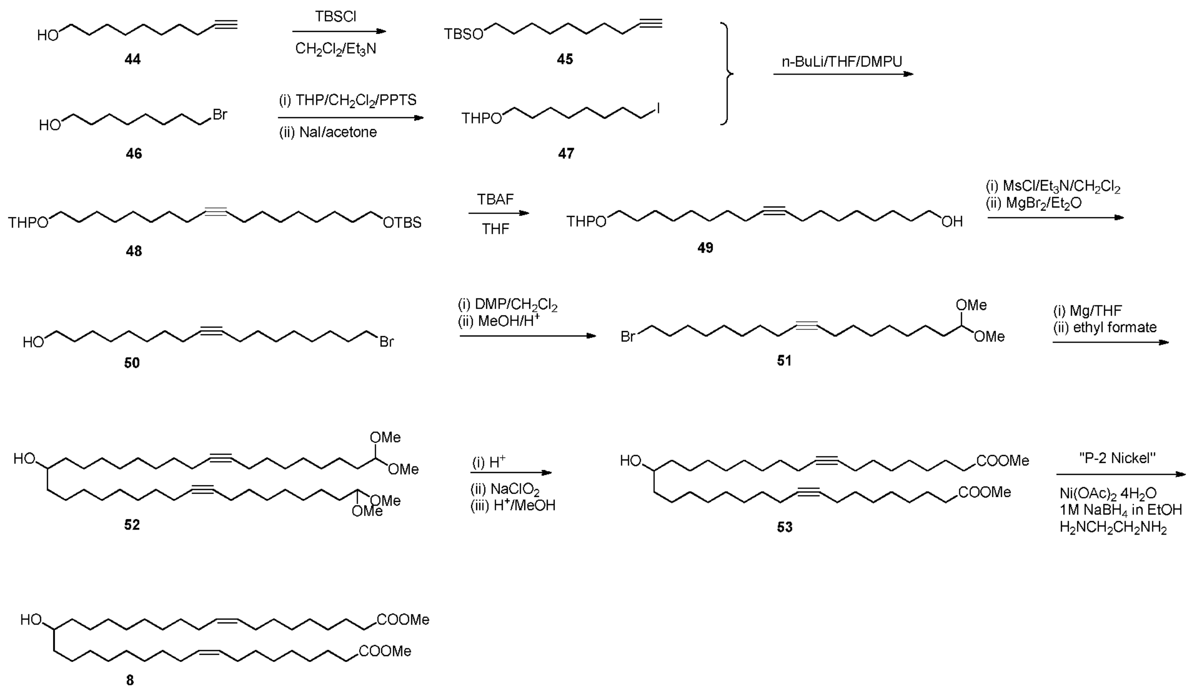
0.25 developed with CH_2Cl_2:MeOH:AcOH = 95:4.5:0.5). Molecular weight for $C_{43}H_{77}O_5Si$ (M-H)$^-$ Calc. 701.5540, Found 701.5. This compound can be synthesized by TEMPO oxidation.

Compound **42**: A procedure analogous to that described for compound **19** afforded compound **42** (23 mg 0.032 mmol, 21 % from compound **40**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 6 H), 3.59-3.62 (m, 1 H), 2.30 (t, J = 7.5 Hz, 4 H), 2.13 (t, J = 6.8 Hz, 8 H), 1.27-1.64 (m, 48 H), 0.88 (s, 9 H), 0.03 (s, 6 H).

Reduction using P-2 nickel conditions can give compound **43** and subsequent deprotection by TBAF can afford compound **8**.

Example 5: Alternate Synthesis for Compound 8

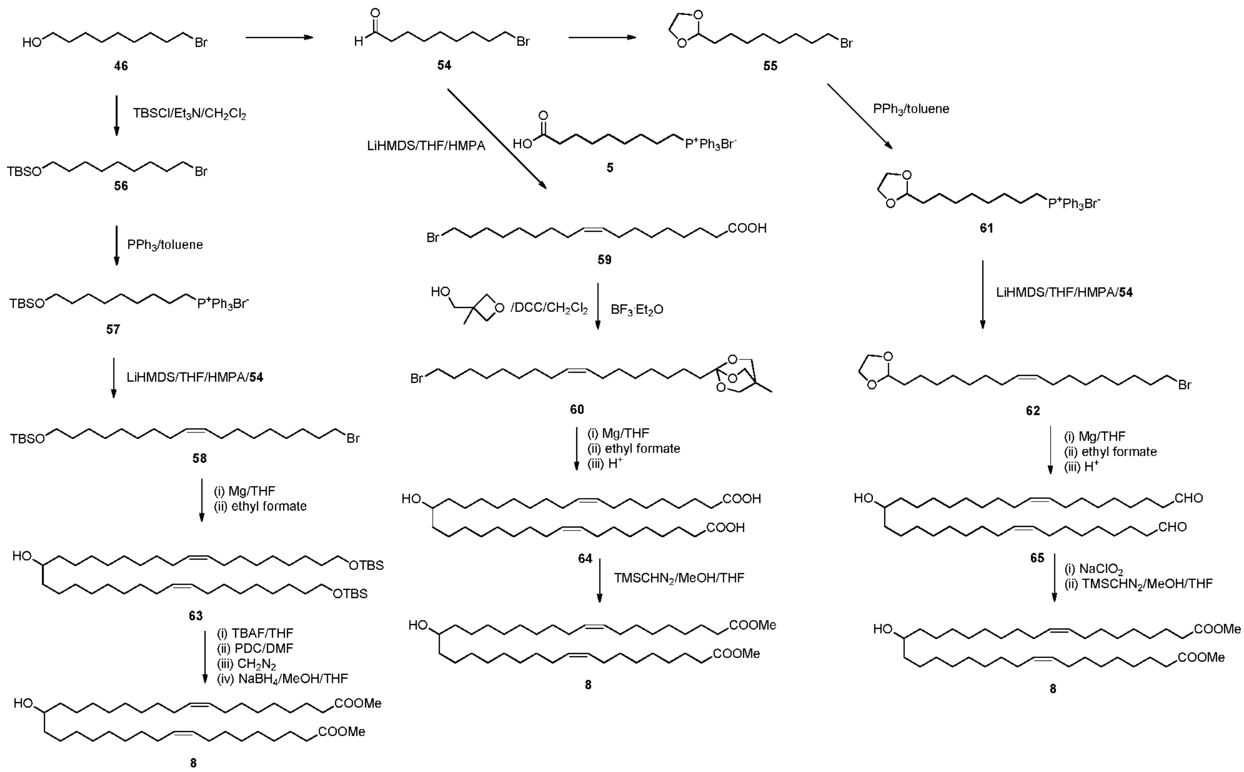
Scheme 5



Compound **8** can be synthesized as shown in Scheme 5. The bromide **51** can be converted to its Grignard reagent then coupled with ethyl formate to afford compound **52**. Subsequent acid treatment, oxidation, and reduction can give compound **8**.

Example 6: Alternate Synthesis for Compound 8

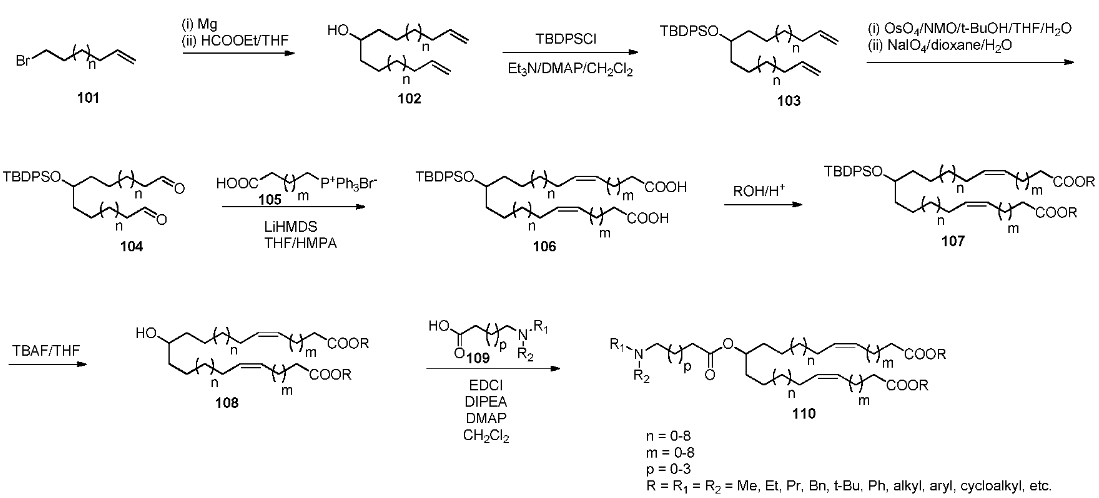
Scheme 6



Compound **8** can be synthesized as shown in Scheme 6. Either bromides of compound **58**, **60**, or **62** can be reacted with ethyl formate to generate terminal-functionalized di-olefin chain. Compound 8 can then be prepared from the diolefin chain compounds using standard chemical reactions.

Example 7: General synthetic scheme for terminal ester lipids

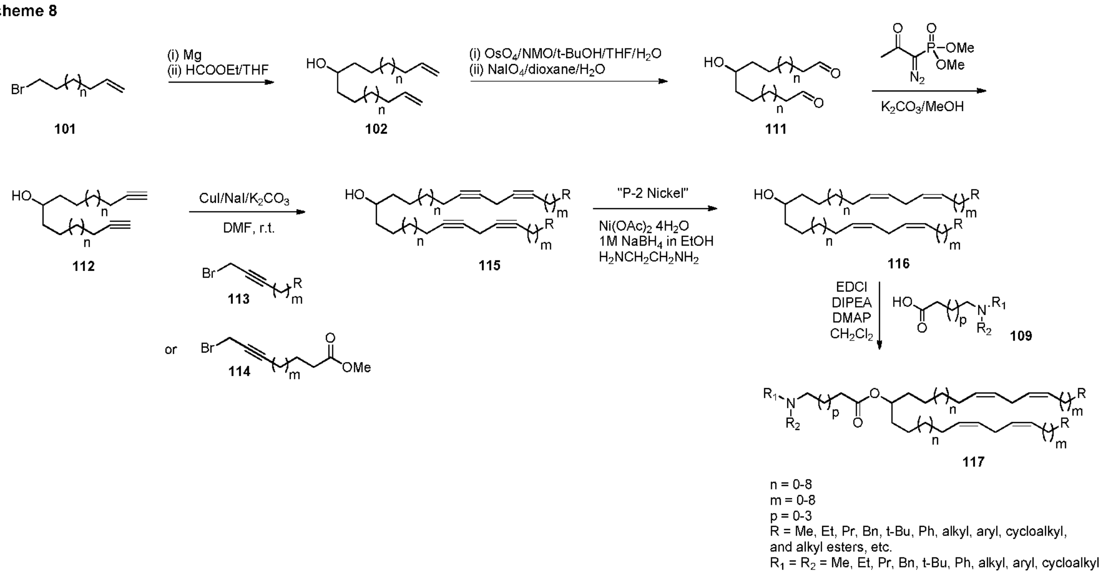
Scheme 7



As shown in **Scheme 7**, chain length and linker length as well as alkyl groups in ester functionality and substituents on nitrogen atom can be derivatized.

Example 8: General synthetic scheme 2 for terminal ester lipids

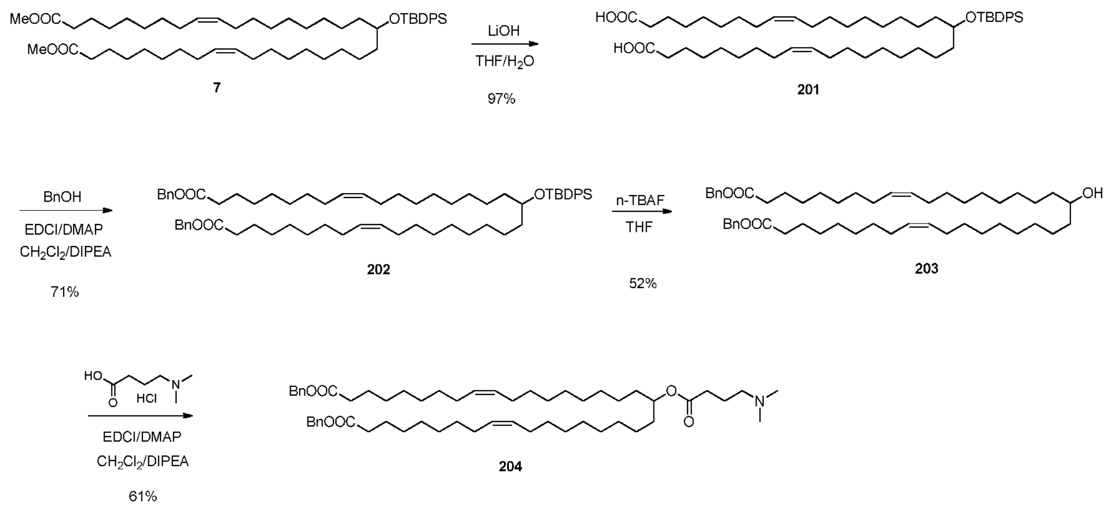
Scheme 8



As shown in **Scheme 8**, copper-mediated coupling affords di-yne containing lipid chain with terminal functional groups, which can be easily reduced to generate di-ene containing lipid chains. The length of linker and lipid chain as well as functional substituent groups (R, R_1, R_2) can be derivatized.

Example 9: Synthesis of terminal benzyl ester lipid

Scheme 9



Compound **201**: Compound **7** (1.30 g, 1.51 mmol) was treated with lithium hydroxide monohydrate (317 mg, 7.55 mmol) in THF (25 mL) and H_2O (5 mL) for 12 h. Amberlite IR-120 (plus) ion exchange resin was added then stirred for 10 minutes. The resulting clear solution was filtered, washed with THF/H_2O and evaporated. Co-evaporation with toluene gave the compound **201** (1.22 g, 1.47 mmol, 97%). Molecular weight for $C_{53}H_{85}O_5Si$ $(M-H)^-$ Calc. 829.6166, Found 829.5.

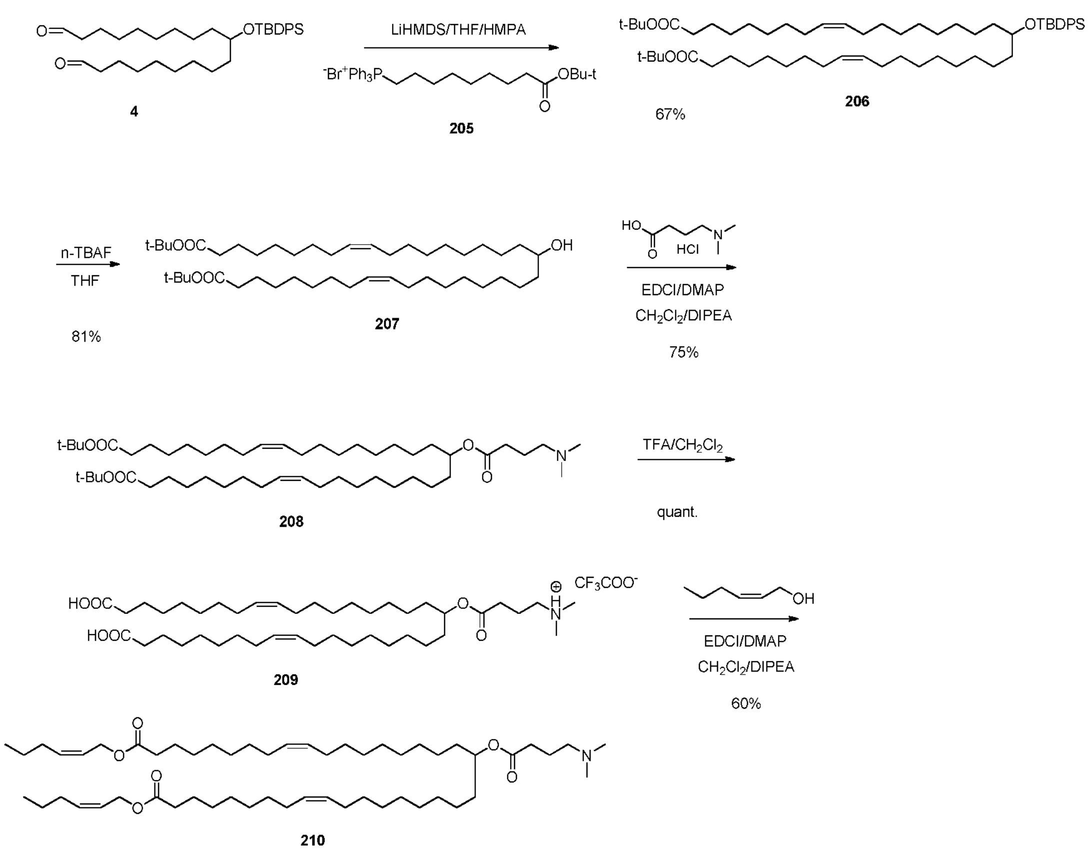
Compound **202**: A procedure analogous to that described for compound **9** was followed with benzylalcohol and **201** (101 mg, 0.121 mmol) to afford compound **202** (87 mg, 0.0860 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.66 (m, 4 H), 7.42 – 7.30 (m, 16 H), 5.38 – 5.30 (m, 4 H), 5.11 (s, 4 H), 3.71 – 3.68 (m, 1 H), 2.35 (t, J = 7.6 Hz, 4 H), 2.04 – 1.97 (m, 8 H), 1.66 – 1.62 (m, 4 H), 1.40 – 1.07 (m, 44 H), 1.04 (s, 9 H).

Compound **203**: A procedure analogous to that described for compound **8** was followed with **202** (342 mg, 0.338 mmol) to afford compound **202** (136 mg, 0.176 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 10 H), 5.38 – 5.30 (m, 4 H), 5.11 (s, 4 H), 3.57 (brs, 1 H), 2.35 (t, J = 7.6 Hz, 4 H), 2.01 – 1.98 (m, 8 H), 1.66 – 1.60 (m, 4 H), 1.45 – 1.25 (m, 44 H).

Compound **204**: A procedure analogous to that described for compound **9** was followed with **203** (133 mg, 0.172 mmol) to afford compound **204** (93 mg, 0.105 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 10 H), 5.38 – 5.30 (m, 4 H), 5.11 (s, 4 H), 4.88 – 4.83 (m, 1 H), 2.37 – 2.27 (m, 8 H), 2.22 (s, 6 H), 2.03 – 1.97 (m, 8 H), 1.81 – 1.26 (m, 50 H).

Example 10: Synthesis of terminal t-butyl ester lipid and the derivatives

Scheme 10



Compound **206**: A procedure analogous to that described for compound **12** was followed with **205** (3.80 g, 0.7.61 mmol) and **4** (1.75 g, 3.17 mmol) to afford compound **206** (2.00 g, 2.12 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.66 (m, 4 H), 7.42 – 7.33 (m, 6 H), 5.39 – 5.31 (m, 4 H), 3.71 – 3.68 (m, 1 H), 2.20 (t, J = 7.6 Hz, 4 H), 2.01 – 1.98 (m, 8 H), 1.59 – 1.55 (m, 4 H), 1.44 (s, 18 H), 1.41 – 1.11 (m, 44 H), 1.04 (s, 9 H).

Compound **207**: A procedure analogous to that described for compound **8** was followed with **206** (265 mg, 0.281 mmol) to afford compound **207** (161 mg, 0.228 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 – 5.30 (m, 4 H), 3.58 (brs, 1 H), 2.20 (t, J = 7.4 Hz, 4 H), 2.01 – 1.98 (m, 8 H), 1.59 – 1.55 (m, 4 H), 1.44 (s, 18 H), 1.35 – 1.26 (m, 44 H).

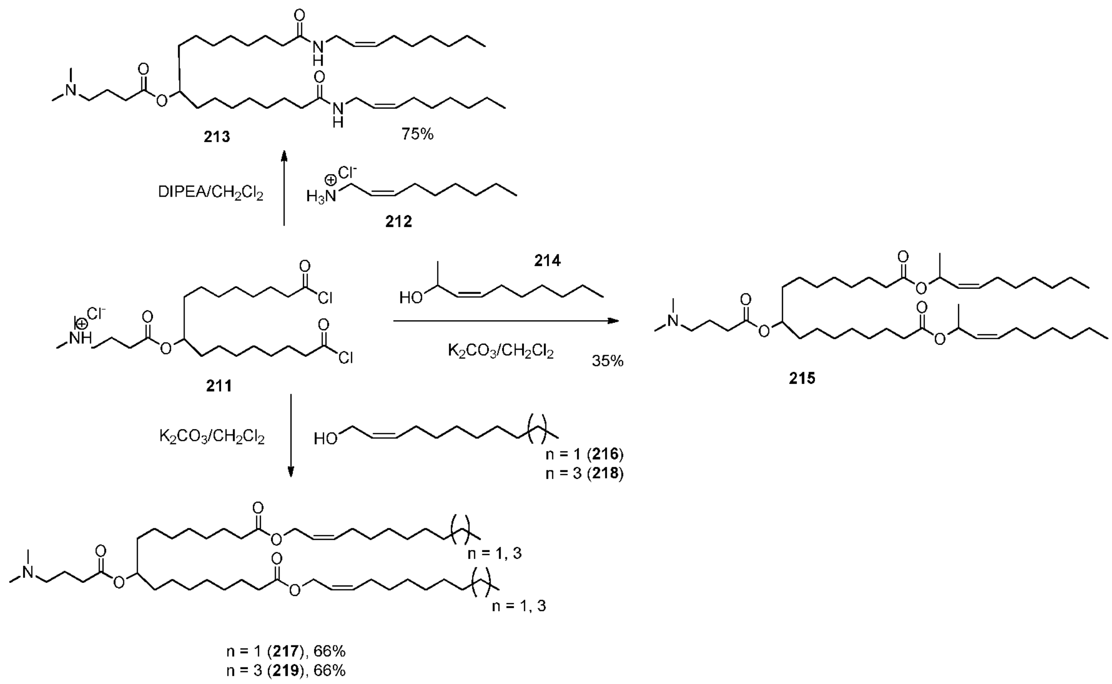
Compound **208**: A procedure analogous to that described for compound **9** was followed with **207** (158 mg, 0.224 mmol) to afford compound **208** (138 mg, 0.169 mmol, 75%). Molecular weight for $C_{51}H_{96}NO_6$ $(M+H)^+$ Calc. 818.7238, Found 818.7.

Compound **209**: Compound **208** (148 mg, 0.181 mmol) was treated with TFA (1.5 mL) in CH_2Cl_2 (6 mL) for 2.5 h. After evaporation and co-evaporation with toluene gave the compound **209** (154 mg, quant.). Molecular weight for $C_{43}H_{80}NO_6$ $(M+H)^+$ Calc. 706.5980, Found 706.5.

Compound **210**: A procedure analogous to that described for compound **9** was followed with **209** (0.061 mmol) and *cis*-2-Hexen-1-ol (18.3 mg, 0.183 mmol) to afford compound **210** (32 mg, 0.0368 mmol, 60%). Molecular weight for $C_{55}H_{100}NO_6$ $(M+H)^+$ Calc. 870.7551, Found 870.5.

Example 11: Synthesis of internal ester/amide lipids-1

Scheme 11



Compound **213**: Compound **211** (503 mg, 1.0 mmol) was treated with **212** (533 mg, 3.0 mmol) in CH_2Cl_2 (35 mL) and DIPEA (1.74 mL, 10 mmol) for 14 h. Aqueous work-up then column chromatography gave compound **213** (506 mg, 0.748 mmol, 75%). Molecular weight for $C_{41}H_{78}N_3O_4$ $(M+H)^+$ Calc. 676.5992, Found 676.4.

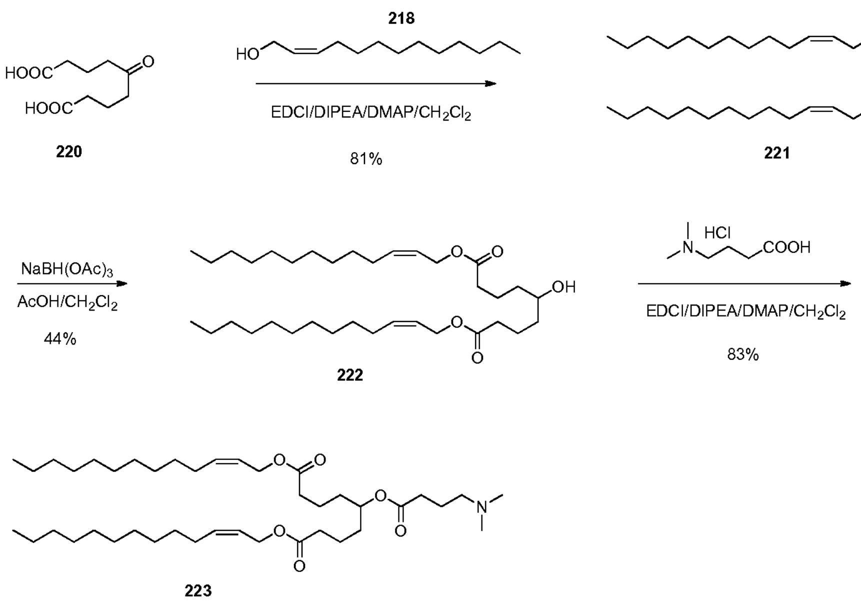
Compound **215**: Compound **211** (503 mg, 1.0 mmol) was treated with **214** (469 mg, 3.0 mmol) and K_2CO_3 (1.38 g, 10 mmol) in CH_2Cl_2 (35 mL) for 14 h. Aqueous work-up then column chromatography gave compound **215** (244 mg, 0.346 mmol, 35%). Molecular weight for $C_{43}H_{80}NO_6$ $(M+H)^+$ Calc. 706.5986, Found 706.4.

Compound **217**: Compound **211** (425 mg, 0.845 mmol) was treated with **216** (525 mg, 3.08 mmol) and K_2CO_3 (1.17 g, 8.45 mmol) in CH_2Cl_2 (35 mL) for 14 h. Aqueous work-up then column chromatography gave compound **217** (407 mg, 0.554 mmol, 66%). Molecular weight for $C_{45}H_{84}NO_6$ $(M+H)^+$ Calc. 734.6299, Found 734.4.

Compound **219**: Compound **211** (503 mg, 1.0 mmol) was treated with **218** (595 mg, 3.0 mmol) and K_2CO_3 (1.38 g, 10 mmol) in CH_2Cl_2 (35 mL) for 14 h. Aqueous work-up then column chromatography gave compound **219** (519 mg, 0.657 mmol, 66%). Molecular weight for $C_{49}H_{92}NO_6$ $(M+H)^+$ Calc. 790.6925, Found 790.7.

Example 12: Synthesis of internal ester lipid-223

Scheme 12



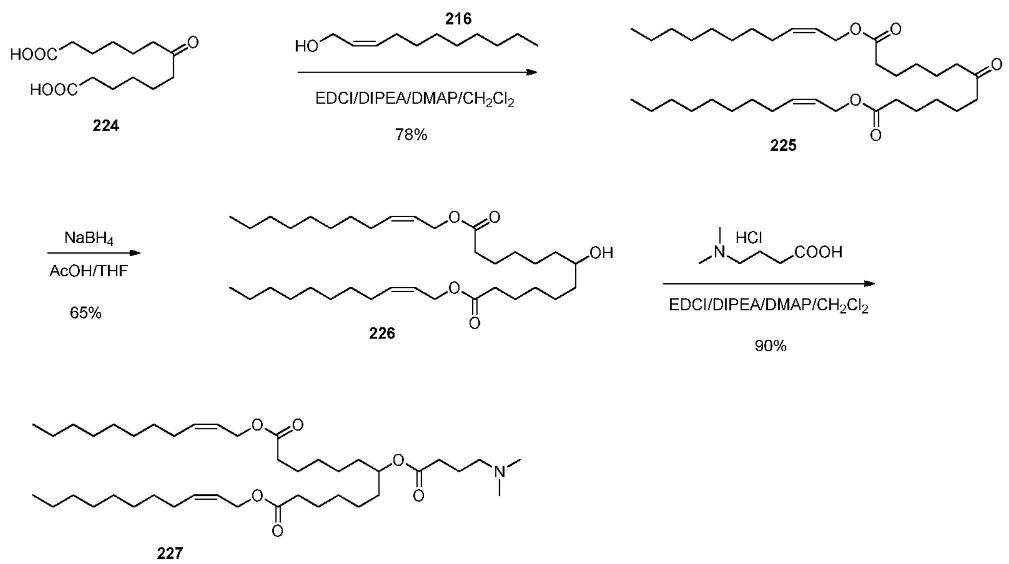
Compound **221**: A procedure analogous to that described for compound **9** was followed with **220** (390 mg, 1.93 mmol) and **218** (765 mg, 3.86 mmol) to afford compound **221** (878 mg, 1.56 mmol, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.67 – 5.61 (m, 2 H), 5.54 – 5.48 (m, 2 H), 4.62 (d, J = 6.8 Hz, 4 H), 2.47 (t, J = 7.2 Hz, 4 H), 2.33 (t, J = 7.2 Hz, 4 H), 2.12 – 2.06 (m, 4 H), 1.93 – 1.86 (m, 4 H), 1.38 – 1.26 (m, 32 H), 0.88 (t, J = 6.8 Hz, 6 H).

Compound **222**: Compound **221** (318 mg, 0.565 mmol) was treated with $NaBH(OAc)_3$ (360 mg, 1.70 mmol) in CH_2Cl_2 (5 mL) and AcOH (0.2 mL) for 16 h. After evaporation, column chromatography gave compound **222** (141 mg, 0.250 mmol, 44%). Molecular weight for $C_{35}H_{65}O_5$ $(M+H)^+$ Calc. 565.4832, Found 565.4.

Compound **223**: A procedure analogous to that described for compound **9** was followed with **222** (137 mg, 0.243 mmol) to afford compound **223** (137 mg, 0.202 mmol, 83%). Molecular weight for $C_{41}H_{76}NO_6$ (M+H)$^+$ Calc. 678.5673, Found 678.5.

Example 13: Synthesis of internal ester lipid-227

Scheme 13



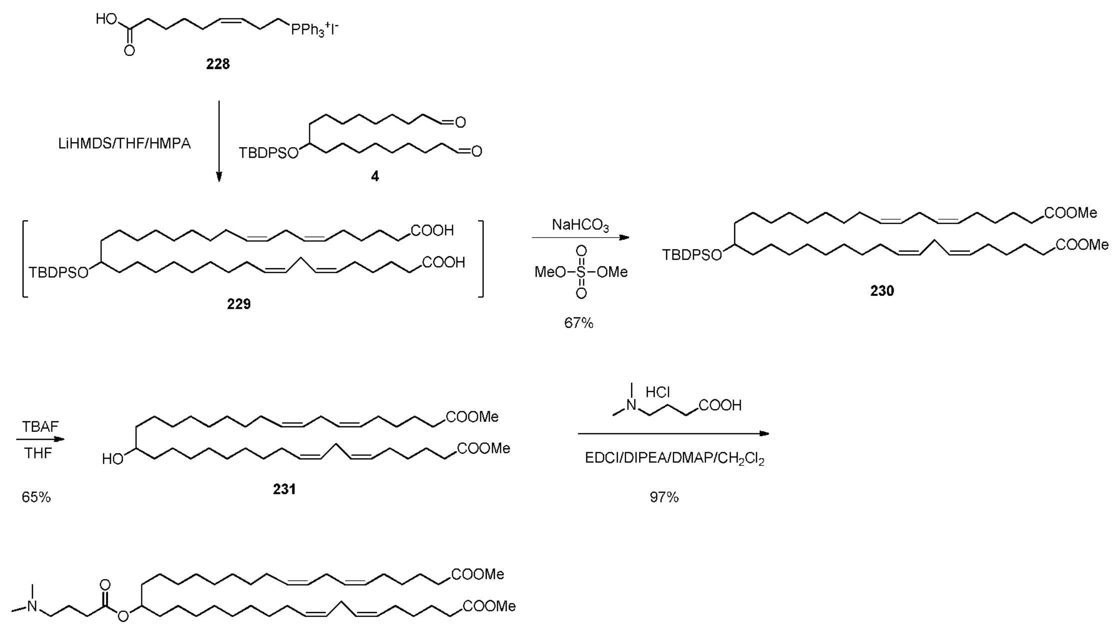
Compound **225**: A procedure analogous to that described for compound **9** was followed with **224** (200 mg, 0.774 mmol) and **216** (264 mg, 1.55 mmol) to afford compound **225** (341 mg, 0.606 mmol, 78%). Molecular weight for $C_{35}H_{62}NaO_5$ (M+Na)$^+$ Calc. 585.4495, Found 585.5.

Compound **226**: Compound **225** (283 mg, 0.503 mmol) was treated with $NaBH_4$ (57 mg, 1.51 mmol) in THF (5 mL) and AcOH (0.2 mL) for 8 h. After evaporation, column chromatography gave compound **226** (185 mg, 0.328 mmol, 65%). Molecular weight for $C_{35}H_{64}NaO_5$ (M+Na)$^+$ Calc. 587.4651, Found 587.3.

Compound **227**: A procedure analogous to that described for compound **9** was followed with **226** (230 mg, 0.407 mmol) to afford compound **227** (248 mg, 0.366 mmol, 90%). Molecular weight for $C_{41}H_{76}NO_6$ $(M+H)^+$ Calc. 678.5673, Found 678.5.

Example 14: Synthesis of terminal ester lipid with linoleyl chain-232

Scheme 14



232

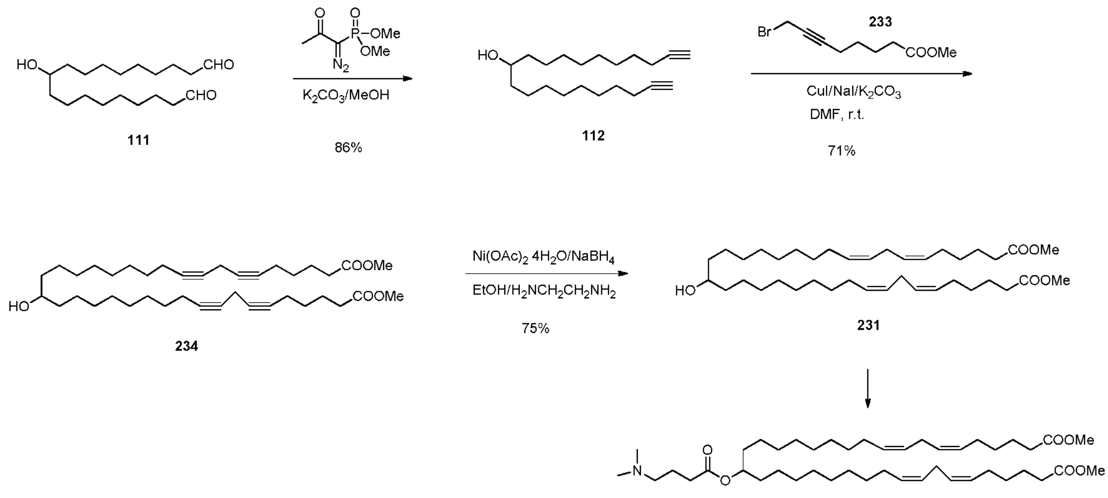
Compound **230**: A procedure analogous to that described for compound **7** was followed with **228** (3.27 g, 6.0 mmol) and **4** (1.27 g, 2.30 mmol) to afford compound **230** (1.31 g, 1.53 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.66 (m, 4 H), 7.42 – 7.33 (m, 6 H), 5.42 – 5.29 (m, 8 H), 3.71 – 3.68 (m, 1 H), 3.66 (s, 6 H), 2.77 (t, J = 5.8 Hz, 4 H), 2.33 – 2.28 (m, 4 H), 2.11 – 2.01 (m, 8 H), 1.69 – 1.60 (m, 4 H), 1.43 – 1.10 (m, 32 H), 1.04 (s, 9 H).

Compound **231**: A procedure analogous to that described for compound **8** was followed with **230** (1.30 g, 1.52 mmol) to afford compound **231** (611 mg, 0.990 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 – 5.29 (m, 8 H), 3.67 (s, 6 H), 3.58 (brs, 1 H), 2.77 (t, J = 5.8 Hz, 4 H), 2.32 (t, J = 7.4 Hz, 4 H), 2.10 – 2.00 (m, 8 H), 1.69 – 1.60 (m, 4 H), 1.43 – 1.29 (m, 32 H).

Compound **232**: A procedure analogous to that described for compound **9** was followed with **231** (520 mg, 0.843 mmol) to afford compound **232** (600 mg, 0.822 mmol, 97%). Molecular weight for $C_{45}H_{80}NO_6$ $(M+H)^+$ Calc. 730.5986, Found 730.5.

Example 15: Synthesis of terminal ester lipid with linoleyl chain-232

Scheme 15



Compound **231** was also synthesized as shown Scheme 15.

Compound **112**: Compound **111** (840 mg, 2.69 mmol) was treated with dimethyl (1-diazo-2-oxopropyl)phosphonate (0.970 mL, 6.46 mmol) and K_2CO_3 (1.49 g, 10.8 mmol) in MeOH (40 mL) for 6 h. Aqueous work-up then column chromatography gave compound **112** (700 mg, 2.30 mmol, 86%). ^1H NMR (400 MHz, $CDCl_3$) δ 3.58 (brs, 1 H), 2.18 (td, J = 7.1, 2.6 Hz, 4 H), 1.94 (t, J = 2.6 Hz, 2 H), 1.56 – 1.25 (m, 28 H).

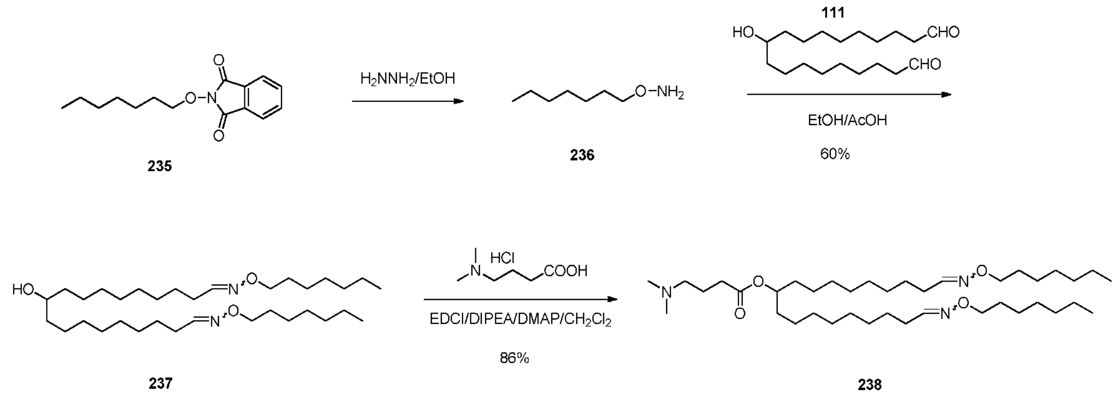
Compound **234**: Compound **112** (207 mg, 0.680 mmol) was treated with **233** (316 mg, 1.36 mmol), K_2CO_3 (282 mg, 2.04 mmol), NaI (408 mg, 2.72 mmol) and CuI (518 mg, 2.72 mmol) in DMF (3.5 mL) for 18 h. Aqueous work-up then column chromatography gave compound **234**

(292 mg, 0.480 mmol, 71%). Molecular weight for $C_{39}H_{61}O_5$ $(M+H)^+$ Calc. 609.4519, Found 609.5.

Compound **231**: To a stirred solution of nickel(II) acetate tetrahydrate (533 mg, 2.14 mmol) in EtOH (28.5 mL), 1 M solution of $NaBH_4$ in EtOH (2.14 mL) was added at room temperature. After 30 min, ethylenediamine (0.574 mL, 8.57 mmol) and a solution of **234** (290 mg, 0.476 mmol) in EtOH (3 mL) was added then stirred for 1 h. The reaction mixture was filtered through Celite and evaporated. Aqueous work-up then column chromatography gave compound **231** (219 mg, 0.355 mmol, 75%). Molecular weight for $C_{39}H_{69}O_5$ $(M+H)^+$ Calc. 617.5145, Found 617.3.

Example 16: Synthesis of internal oxime lipid-238

Scheme 16

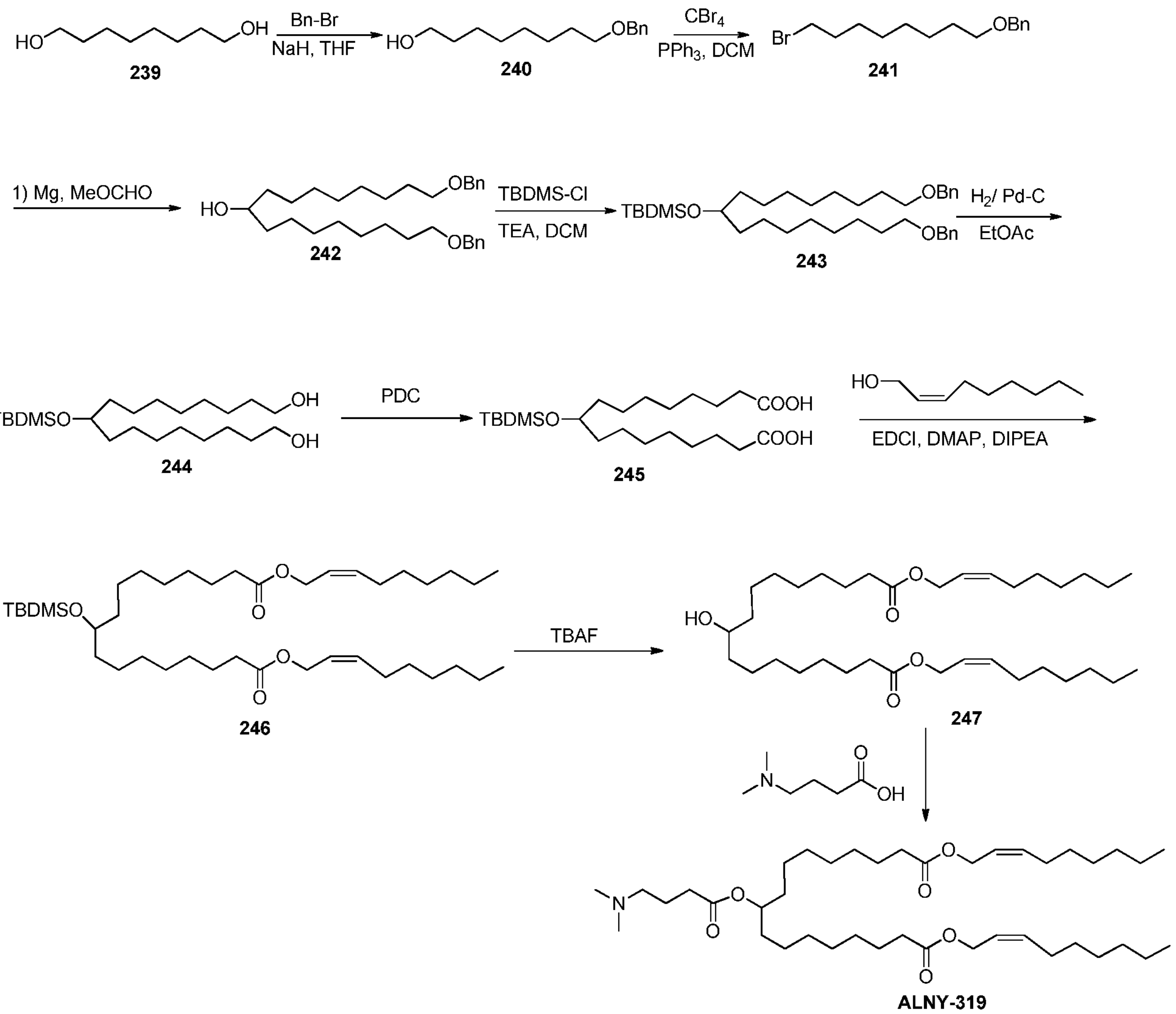


Compound **237**: Compound **235** (465 mg, 1.78 mmol) was treated with hydrazine monohydrate (64-65%, 0.135 mL, 1.78 mmol) in EtOH (15 mL) for 4 h. After filtration then evaporation, the crude was re-suspended in EtOH (5 mL). To this solution was added compound **111** (160 mg, 0.512 mmol) and AcOH (a few drops). Aqueous work-up then column chromatography gave compound **237** (165 mg, 0.306 mmol, 60%). Molecular weight for $C_{33}H_{67}N_2O_3$ $(M+H)^+$ Calc. 539.5152, Found 539.3.

Compound **238**: A procedure analogous to that described for compound **9** was followed with **237** (162 mg, 0.301 mmol) to afford compound **238** (168 mg, 0.258 mmol, 86%). Molecular weight for $C_{39}H_{78}N_3O_4$ $(M+H)^+$ Calc. 652.5992, Found 652.4.

Example 17

Scheme 17:



8-benzyloxy-octan-1-ol (240): To a stirred suspension of NaH (60% in oil, 82 g, 1.7096 mol) in 500mL anhydrous DMF, a solution of compound **239** (250g, 1.7096 mol) in 1.5 L DMF was added slowly using a dropping funnel at 0° C. The reaction mixture was stirred for 30 minutes, then benzyl bromide (208.86 mL, 1.7096 mol) was added slowly under a nitrogen atmosphere. The reaction was then warmed to ambient temperature and stirred for 10 hours. After completion of reaction, the mixture was quenched with crushed ice (~2kg) and extracted with ethyl acetate (2 x 1L). The organic layer washed with water (1L) to remove unwanted DMF, dried over

Na_2SO_4 and evaporated to dryness under vacuum. The crude compound was purified on 60-120 silica gel, eluted with 0-5% MeOH in DCM to afford compound **240** (220g, 54%) as pale yellow liquid. H^1 NMR (400MHz, $CDCl_3$): δ = 7.33-7.24 (m, 5H), 4.49 (s, 2H), 3.63-3.60 (m, 2H), 3.47-3.43 (m, 2H), 1.63-1.51 (m, 4H), 1.39-1.23 (m, 8H).

(8-bromo-octyloxymethyl)-benzene (241): Compound **240** (133g, 0.5635mol) was dissolved in 1.5 L of DCM, CBr_4 (280.35g, 0.8456mol) was added to this stirring solution and the reaction mixture was cooled to 0°C under an inert atmosphere. PPh_3 (251.03g, 0.9571mol) was then added in portions maintaining the temperature below 20°C and after complete addition, the reaction mixture was stirred for 3 hours at room temperature. After completion of reaction, solid (PPh_3O) precipitated out from the reaction mixture was isolated by filtrationand the filtrate was diluted with crushed ice (~ 1.5kg) and extracted with DCM (3 x 750mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and distilled under vacuum. The resulting crude compound was chromatographed on 60-120 mesh silica gel column using 0-5 % ethyl acetate in hexanes as eluting system to afford compound **241** (150g, 89%) as pale yellow liquid. ^1H NMR (400MHz, $CDCl_3$): δ = 7.33-7.25 (m, 5H), 4.49 (s, 2H), 3.47-3.41 (m, 2H), 3.41-3.37 (m, 2H), 1.86-1.80 (m, 4H), 1.62-1.56(m, 2H), 1.42-1.29 (m, 8H).

1, 17-bis-benzyloxy-heptadecan-9-ol (242): To freshly activated Mg turnings (24.08g, 1.003mol) was added 200mL anhydrous THF, followed by the addition of pinch of iodine into the mixture under inert atmosphere. After initiation of the Grignard formation a solution of Compound **241** (150g, 0.5016mol) in 1 L of dry THF was added slowly controlling the exothermic reaction. After complete addition, the reaction was heated to reflux for 1 hour, then cooled to room temperature. Methyl formate (60.24g, 1.0033mol) was then added slowly and reaction was continued for 2 hours. After completion, the reaction was quenched by slow addition of 10% HCl followed by water (1 L) and extracted with ethyl acetate (3 x 1L). The organic layer was taken in 5 litre beaker, diluted with 500mL of methanol and cooled to 0°C. To this solution excess of $NaBH_4$ (~ 5eq) was added in portions to ensure the hydrolysis of formate ester which was not cleaved by addition of HCl. The resulting solution was stirred for an hour and then volatilites were removed under vacuum. The residue was taken in water (1 L) and acidified by 10% HCl solution (P^H 4). The product was then extracted with ethyl acetate (3 x 1

L). The organic phase was then dried and concentrated on rotary evaporator to afford compound **242** (57g, 24%) as solid. ^{1}H NMR (400MHz, $CDCl_3$): δ = 7.35-7.32 (m, 8H), 7.29-7.24 (m, 2H), 4.49 (s, 4H), 3.56 (m, 1H), 3.46-3.43 (m, 4H), 1.63-1.56 (m, 4H), 1.44-1.34 (m, 28H). C^{13} NMR (100MHz, $CDCl_3$): δ = 138.56, 128.21, 127.49, 127.34, 72.72, 71.76, 70.37, 37.37, 29.64, 29.56, 29.47, 29.33, 26.07, 25.54.

[9-benzyloxy-1-(8-benzylozy-octyl)-nonyloxy]-*tert*-butyl-dimethyl-silane (243): Compound **242** (56 g, 0.1196 mol) was dissolved in 700 mL of anhydrous THF and cooled to 0 °C. TBMS-Cl (36.06g, 0.2396mol) was added slowly followed by addition of imidazole (32.55 g, 0.4786 mol) under an inert atmosphere. The reaction was then stirred at room temperature for 18 hours, then quenched with ice (~1kg). The product was extracted with ethyl acetate (3 x 500mL). The organic layer was separated, washed with saturated $NaHCO_3$ solution to remove the acidic impurity, dried over Na_2SO_4 and evaporated under reduce pressure to obtain crude compound which was purified by silica gel (60-120 mesh) and eluted with 0- 10% ethyl acetate hexane to afford (60g, 82%) of compound **243** as yellowish oil. H^1 NMR (400MHz, $CDCl_3$): δ = 7.33-7.24 (m, 10H), 4.49 (s, 4H), 3.60-3.57 (m, 1H), 3.46-3.43 (m, 4H), 1.61-1.54 (m, 4H), 1.41-1.26 (m, 28H), 0.87 (s, 9H), 0.02 (s, 6H)

9-(*tert*-butyl-dimethyl-silanyloxy)-heptadecane-1, 17-diol (244): Compound **243** (60 g, 0.1030 mol) was dissolved in 500mL ethyl acetate and degassed with N_2 for 20 min. (10 wt %) Pd on carbon (12 g) was added and reaction was stirred under an atmosphere of hydrogen for 18 hours. After completion, the mixture was filtered through a bed of celite and washed with ethyl acetate. The filtrate was evaporated under vacuum. Compound **244** (19 g, 46%) thus obtained was pure enough to carry out the next reaction. ^{1}H NMR (400MHz, $CDCl_3$): δ = 3.64-3.58 (m, 5H), 1.59 (br, 2H), 1.57-1.51 (m, 4H), 1.38-1.22 (m, 28H), 0.87 (s, 9H), 0.02 (s, 6H).

9-(*tert*-butyl-dimethyl-silanyloxy)-heptadecanedioic acid (245): To a stirred solution of **244** (2 g, 0.0049 mol) in anhydrous DMF (40 mL) was added pyridinium dirchromate (2.7 g, 0.0074 mol) at 0 °C under an inert atmosphere. The reaction mixture was then allowed to warm to room temperature over a period of 10-15 minutes and continued for 24 hours. Then, the reaction was diluted with water (100mL). The aqueous phase was extracted using DCM (3 x 40mL). The organic phase was washed with brine (1x 25mL) and concentrated under vacuum to afford crude acid which was then purified by (100-200 mesh) silica gel column using 0-30% ethyl acetate in hexanes system. Pure product **(245)** was obtained (0.7g, 33%) as a pale yellow oil. ^{1}H NMR (400MHz, $CDCl_3$): δ = 3.61-3.56 (m, 1H), 2.35-2.32 (m, 4H), 1.64-1.59 (m, 4H), 1.40-1.19 (m, 24H), 0.86 (s, 9H), 0.017 (s, 6H); LC-MS [M+H] - 431.00; HPLC (ELSD) purity – 96.94%

Di((Z)-non-2-en-1-yl) 9-((tert-butyldimethylsilyl)oxy)heptadecanedioate (246): The diacid **245** (0.42 g, 0.97 mmol) was dissolved in 20 mL of dichloromethane and to it *cis*-2-nonen-1-ol (0.35 g, 2.44 mmol) was added followed by Hunig's base (0.68 g, 4.9 mmol) and DMAP (12 mg). To this mixture EDCI (0.47 g, 2.44 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2 (40 mL) and washed with saturated $NaHCO_3$ (50 mL), water (60 mL) and brine (60 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and solvents were removed *in vacuo*. The crude product thus obtained was purified by Combiflash Rf purification system (40 g silicagel, 0-10 % MeOH in CH_2Cl_2) to afford the pure product **246** (0.35 g, 53%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, J = 10.9, 7.4 Hz, 2H), 5.58 – 5.43 (m, 2H), 4.61 (d, J = 6.8 Hz, 4H), 3.71 – 3.48 (m, 1H), 2.30 (t, J = 7.6 Hz, 4H), 2.20 – 1.98 (m, 4H), 1.71 – 1.53 (m, 4H), 1.31 (ddd, J = 8.3, 7.0, 3.7 Hz, 34H), 1.07 – 0.68 (m, 14H), 0.02 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.18, 139.81, 127.78, 81.73, 81.42, 81.10, 76.72, 64.59, 41.52, 41.32, 38.76, 36.09, 34.10, 33.93, 33.80, 33.70, 33.59, 33.55, 33.26, 31.95, 30.34, 29.69, 29.58, 29.39, 27.01, 22.56, 18.48, 0.01.

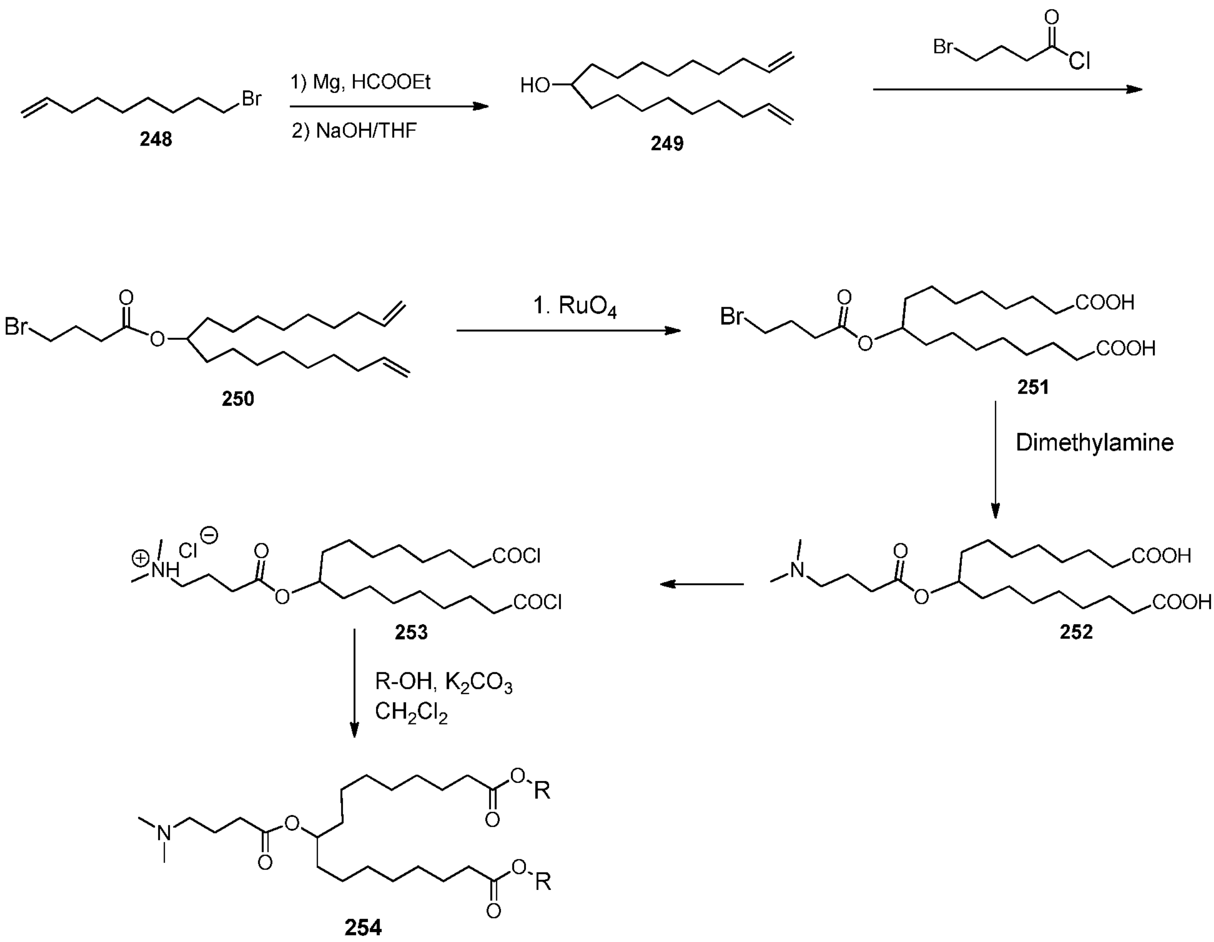
Di((Z)-non-2-en-1-yl) 9-hydroxyheptadecanedioate (247): The silyl protected diester **246** (0.3 g, 0.44 mmol) was dissolved in 1 M solution of TBAF in THF (6 mL) and the solution was kept at 40 °C for two days. The reaction mixture was diluted with water (60 mL) and extracted with ether (2 x 50 mL). The combined organic layers were concentrated and the thus obtained crude product was purified by column to isolate the pure product (0.097 g, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, J = 10.9, 7.4 Hz, 2H), 5.52 (dt, J = 11.0, 6.8 Hz, 2H), 4.61 (d, J = 6.8 Hz, 4H), 3.57 (s, 1H), 2.30 (t, J = 7.5 Hz, 4H), 2.09 (q, J = 7.1 Hz, 4H), 1.75 – 1.53 (m, 4H), 1.53 – 1.06 (m, 36H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.98, 135.64, 123.57, 77.54, 77.22, 76.91, 72.14, 60.41, 37.69, 34.54, 31.89, 29.70, 29.60, 29.44, 29.29, 29.07, 27.76, 25.80, 25.15, 22.82, 14.29.

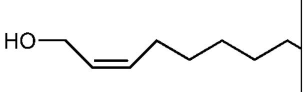
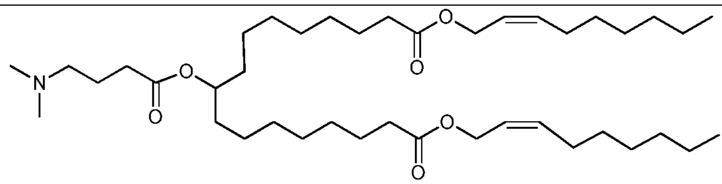
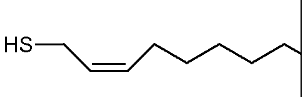
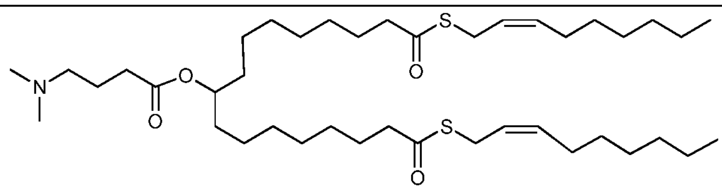
Di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate: The alcohol **247** (0.083 g, 0.147 mmol) was dissolved in 20 mL of dichloromethane and to it dimethylaminobutyric acid hydrochloride (0.030 g, 0.176 mmol) was added followed by Hunig's base (0.045 g, 0.44 mmol) and DMAP (2 mg). To this mixture EDCI (0.034 g, 0.176 mmol) was added and the reaction mixture was stirred at room temperature overnight and the TLC (silica gel, 10% MeOH in CH_2Cl_2) showed complete disappearance of the starting alcohol. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated $NaHCO_3$ (50 mL), water (60 mL) and brine (60 mL). The combined organic layers were dried over anhyd. Na_2SO_4 and solvents were removed *in vacuo*. The crude product thus obtained was purified by Combiflash Rf purification system (40 g silicagel, 0-10 % MeOH in CH_2Cl_2) to isolate the pure product (0.062 g, 62%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 5.74 – 5.58 (m, 2H), 5.51 (dtt, *J* = 9.7, 6.8, 1.3 Hz, 2H), 4.95 – 4.75 (m, 1H), 4.61 (d, *J* = 6.8 Hz, 4H), 2.35 – 2.24 (m, 8H), 2.22 (d, *J* = 7.9 Hz, 6H), 2.09 (q, *J* = 6.9 Hz, 4H), 1.83 – 1.72 (m, 2H), 1.60 (dd, *J* = 14.4, 7.2 Hz, 4H), 1.49 (d, *J* = 5.7 Hz, 4H), 1.41 – 1.13 (m, 30H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.72, 173.36, 135.40, 123.35, 74.12, 60.18, 58.95, 45.46, 34.30, 34.11, 32.45, 31.67, 29.38, 29.35, 29.17, 29.07, 28.84, 27.53, 25.28, 24.93, 23.16, 22.59, 14.06. MW calc. for $C_{41}H_{75}NO_6$ (MH^+): 678.04, found: 678.5.

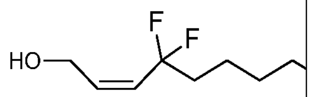
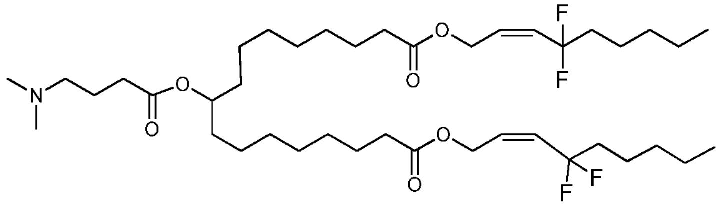
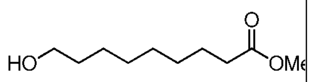
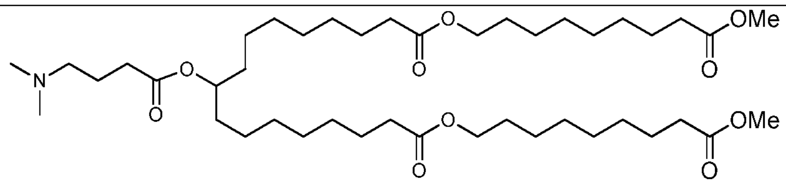
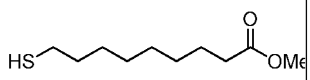
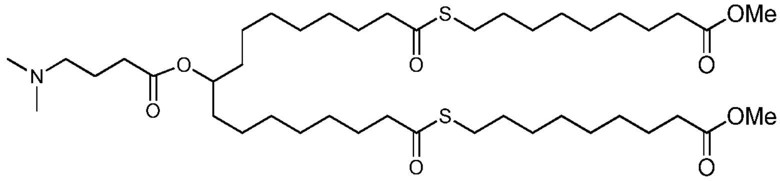
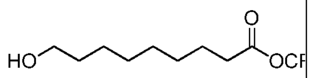
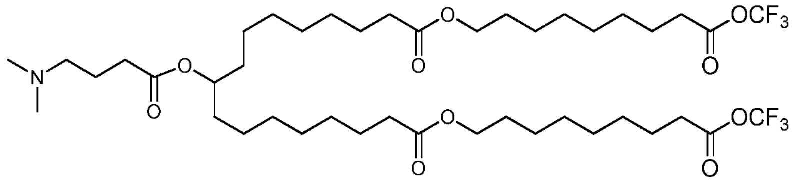
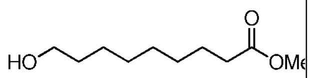
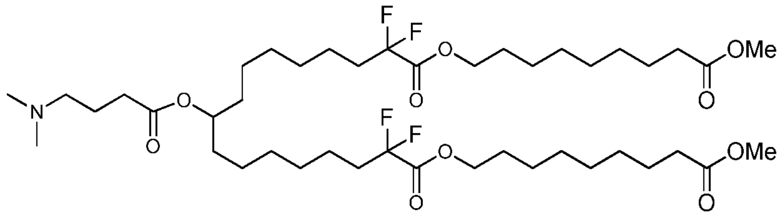
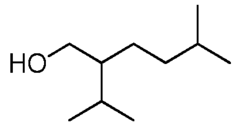
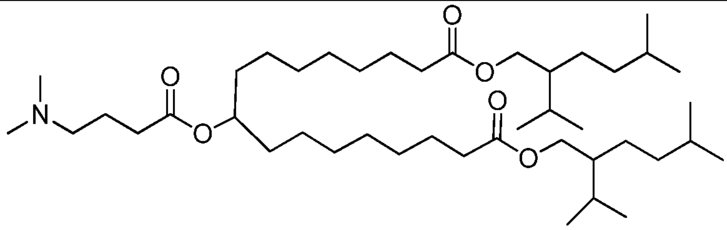
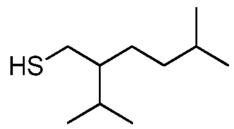
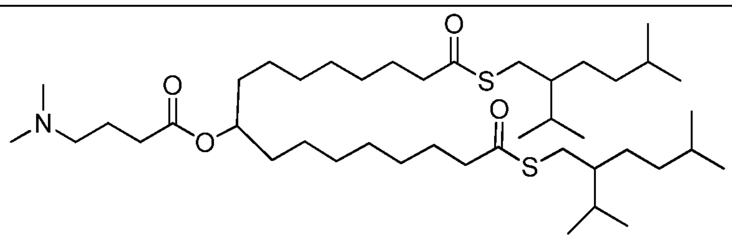
Example 18

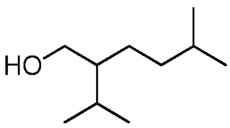
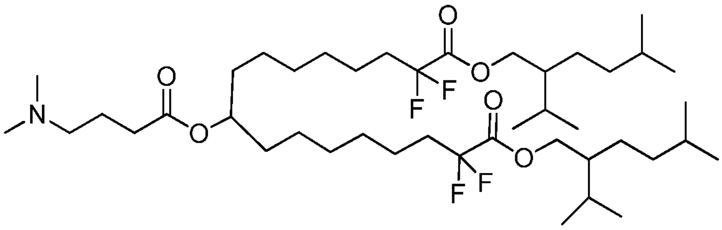
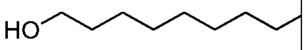
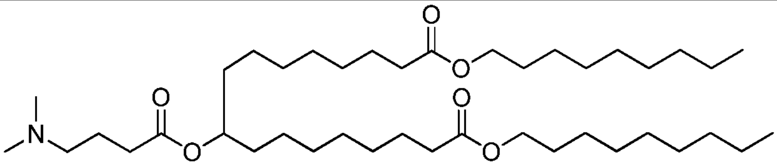
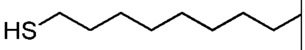
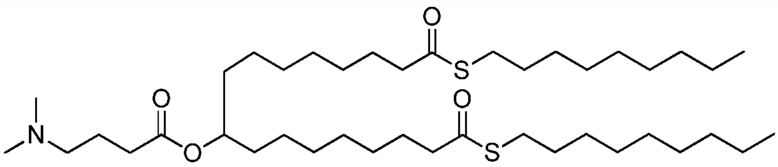
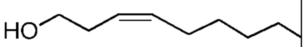
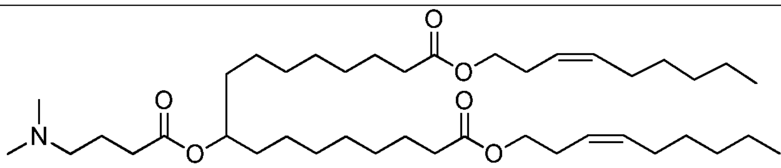
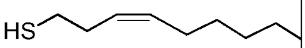
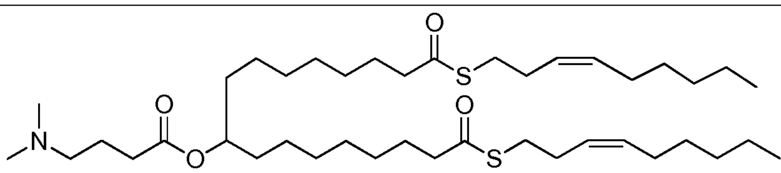
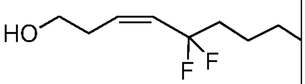
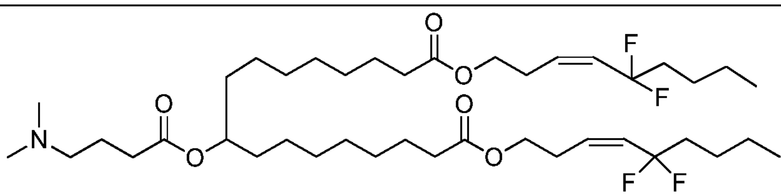
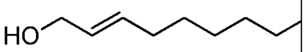
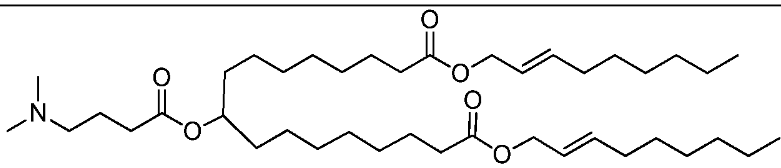
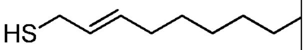
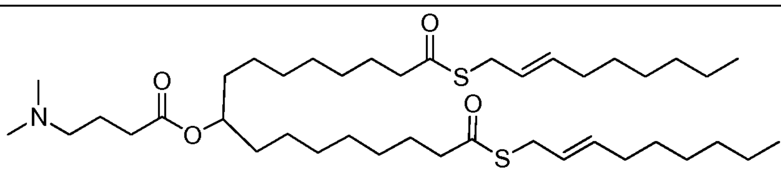
The following shorter route was used for the synthesis of analogs of Compound 1 of the present invention The commercial 9-bromonon-1-ene **248** was treated with magnesium to form the corresponding Grignard reagent which was reacted with ethylformate to give the corresponding adduct **249** which on treatment with bromobutyryl chloride to provide the bromoester **250**. The bromoester **250** on treatment with RuO_4 provided the diacid **251**. The bromodiacid **251** on treatment with dimethylamine provided the amino diacid **252.** The diacid **252** on treatment with oxalyl chloride in the presence of DMF provided the diacid chlorides **253**. The lipids **254a-n** were synthesized by treating the acid Chloride **253** with respective alcohols.

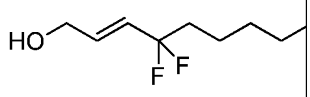
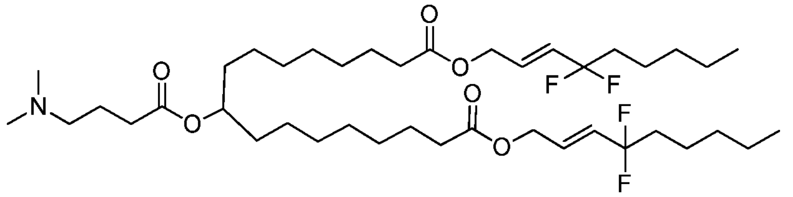
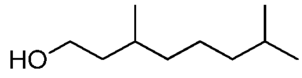
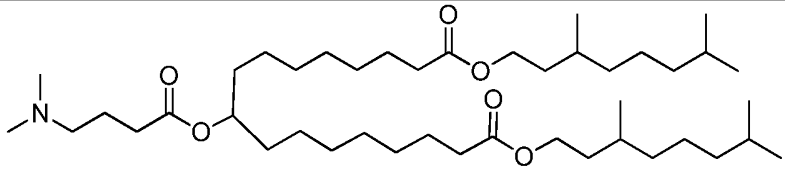
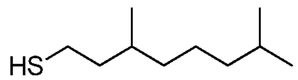
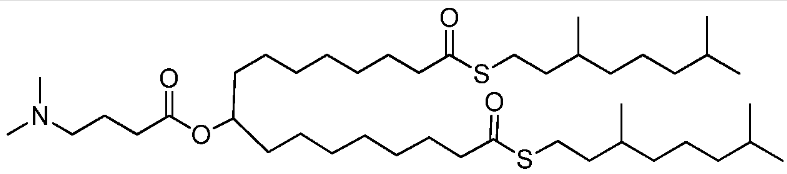
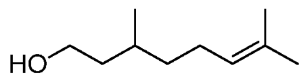
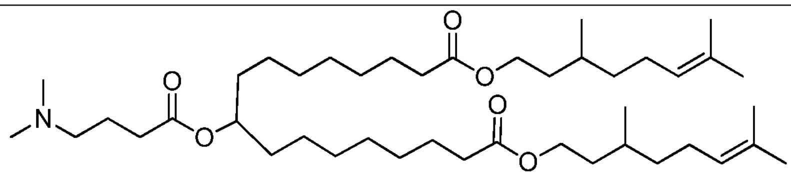
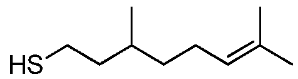
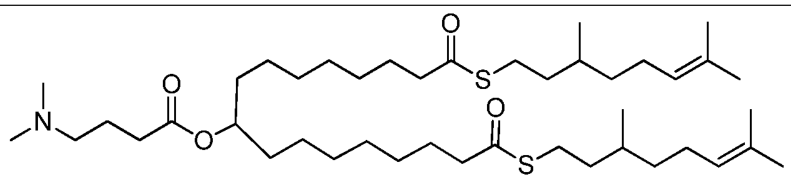
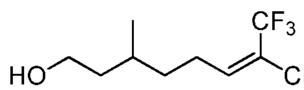
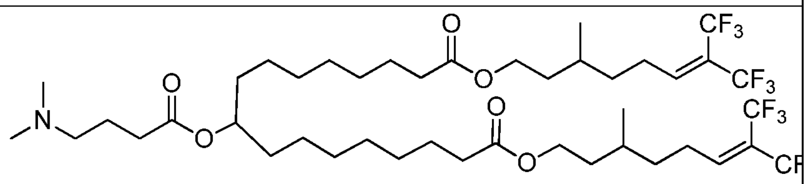
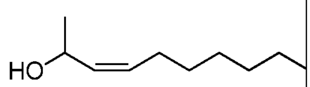
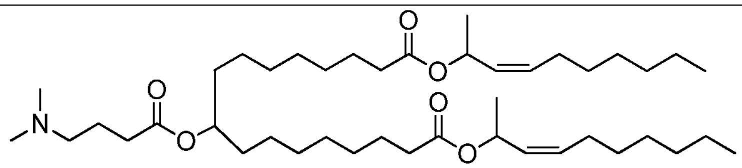
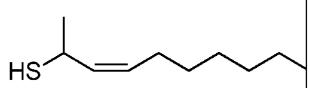
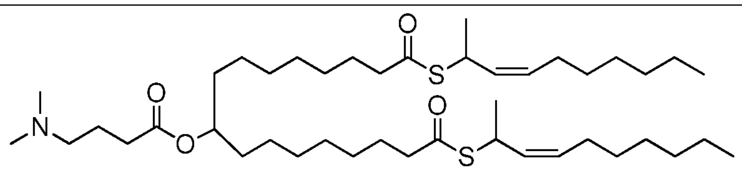
Scheme 18

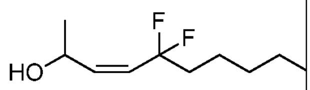
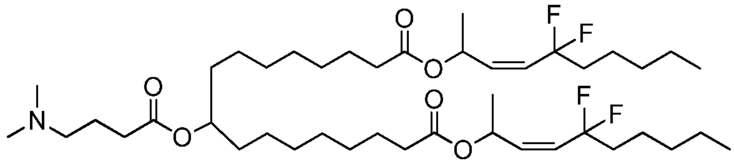
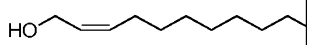
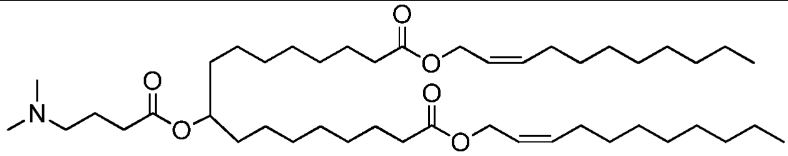
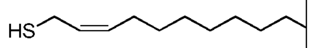
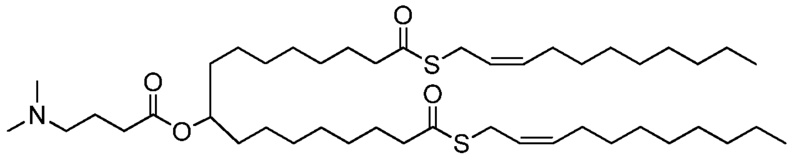
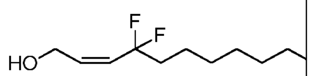
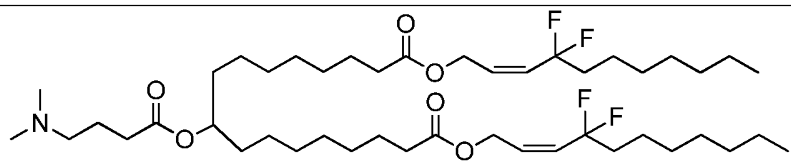
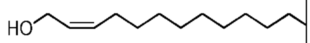
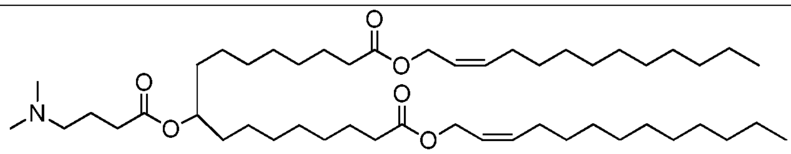
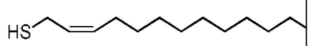
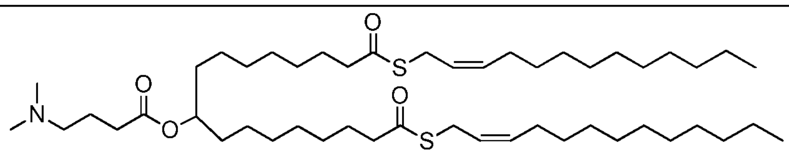
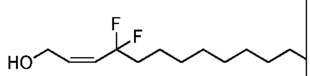
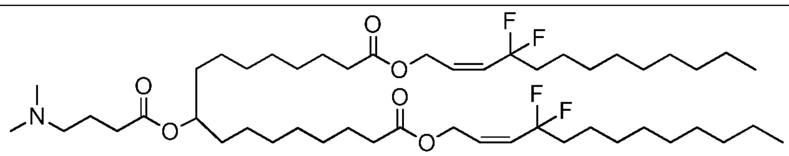
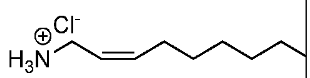
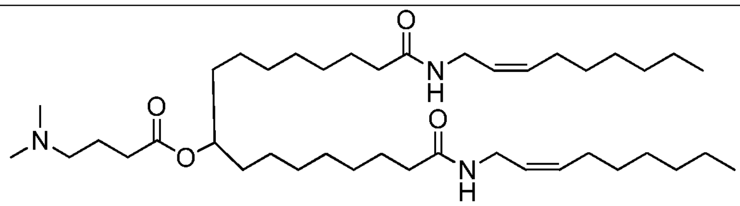
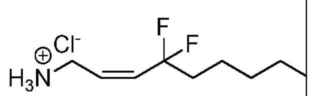
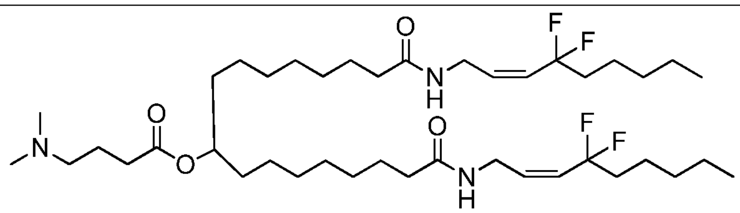


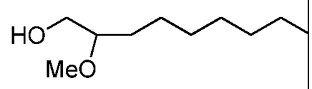
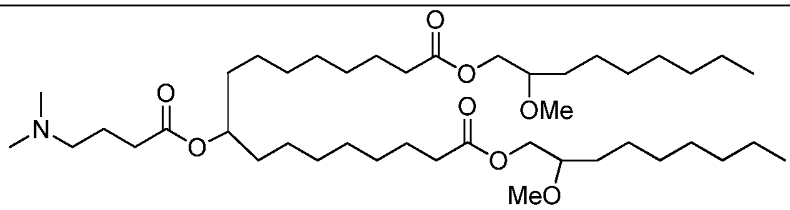
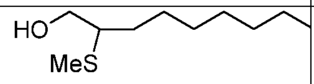
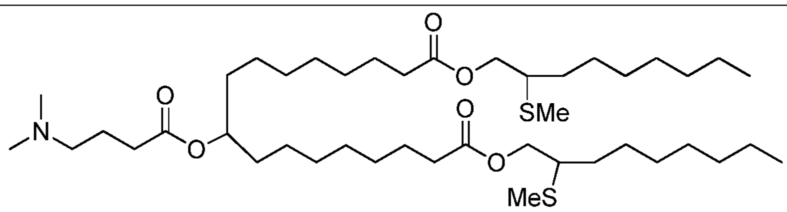
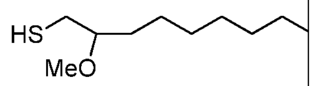
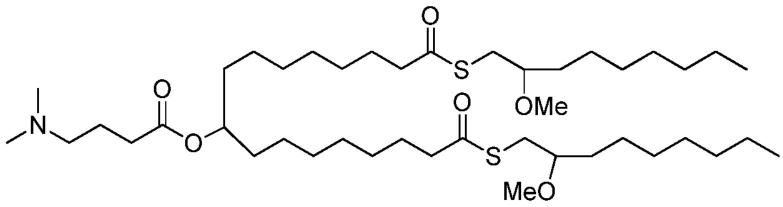
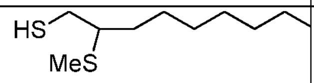
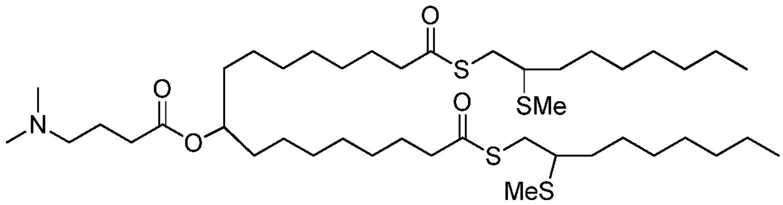
No	Starting Alcohol (ROH)	Product
254a		
254aS		

254aF		
254b		
254bS		
254bF		
254bF2		
254c		
254cS		

254cF		
254d		
254ds		
254e		
254es		
254eF		
254f		
254fs		

254fF		
254g		
254gs		
254h		
254hs		
254hF		
254i		
254is		

254iF		
254j		
254js		
254jF		
254k		
254ks		
254kS		
254l		
254lF		

254m		
254ms		
254ns		
254os		

Synthesis of nonadeca-1,18-dien-10-ol (249)

To a flame dried 500 mL RB flask, freshly activated Mg turnings (9 g) were added and the flask was equipped with a magnetic stir bar, an addition funnel and a reflux condenser. This set-up was degassed and flushed with argon and 100 mL of anhydrous ether was added to the flask via syringe. The bromide **3** (51.3 g, 250 mmol) was dissolved in anhydrous ether (100 mL) and added to the addition funnel. About 5 mL of this ether solution was added to the Mg turnings while stirring vigorously. An exothermic reaction was noticed (to confirm/accelerate the Grignard reagent formation, 5 mg of iodine was added and immediate decolorization was observed confirming the formation of the Grignard reagent) and the ether started refluxing. The rest of the solution of the bromide was added dropwise while keeping the reaction under gentle reflux by cooling the flask in water. After the completion of the addition the reaction mixture was kept at 35 °C for 1 hour and then cooled in ice bath. Ethyl formate (9 g, 121 mmol) was dissolved in anhydrous ether (100 mL) and transferred to the addition funnel and added dropwise to the reaction mixture with stirring. An exothermic reaction was observed and the reaction mixture started refluxing. After the initiation of the reaction the rest of the ethereal solution of formate was quickly added as a stream and the reaction mixture was stirred for a further period of 1 h at ambient temperature. The reaction was quenched by adding 10 mL of acetone dropwise followed by ice cold water (60 mL). The reaction mixture was treated with aq. H_2SO_4 (10 % by volume, 300 mL) until the solution became homogeneous and the layers were separated. The aq. phase was extracted with ether (2x200 mL). The combined ether layers were dried (Na_2SO_4) and concentrated to afford the crude product which was purified by column (silica gel, 0-10% ether in hexanes) chromatography. The product fractions were evaporated to provide the pure product **249** as a white solid (30.6 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 8H), 5.04 – 4.88 (m, 16H), 3.57 (dd, *J* = 7.6, 3.3 Hz, 4H), 2.04 (q, *J* = 6.9 Hz, 16H), 1.59 (s, 1H), 1.45 (d, *J* = 7.5 Hz, 8H), 1.43 – 1.12 (m, 94H), 0.88 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 139.40, 114.33, 77.54, 77.22, 76.90, 72.21, 37.70, 34.00, 29.86, 29.67, 29.29, 29.12, 25.85.

Synthesis of nonadeca-1,18-dien-10-yl 4-bromobutanoate (250)

To a solution of the alcohol **249** (5.6 g, 20 mol) in anhydrous DCM (300 mL) was added slowly and carefully Bromobutryl chloride (20 mmol) at 0° C under inert atmosphere. The reaction mixture was warmed to room temperature, stirred for 20 h and monitored by TLC (silica gel, 10% ethyl acetate in hexanes). Upon completion of the reaction, mixture was diluted with water (400 mL) and organic layer was separated out. Organic phase was then washed with sat. solution of $NaHCO_3$ (1 x 400 mL) followed by brine (1 x 100 mL) and concentrated under vacuum. Crude product was then purified by silica gel (100-200mesh) column, eluted with 2-3% ethyl acetate in hexane solution to give 6 g (90%) of desired product **250** as colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 2H), 5.05 – 4.81 (m, 5H), 3.46 (t, *J* = 6.5 Hz, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 2.17 (p, *J* = 6.8 Hz, 2H), 2.11 – 1.93 (m, 4H), 1.65 – 1.44 (m, 4H), 1.43 – 1.17 (m, 19H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 172.51, 139.37, 114.35, 77.54, 77.23, 76.91, 74.86, 34.31, 33.99, 33.01, 32.96, 29.65, 29.56, 29.24, 29.09, 28.11, 25.52.

Synthesis of 9-((4-bromobutanoyl)oxy)heptadecanedioic acid (251)

To a solution of the bromoester **250** (12.1g, 28.2 mmol) in dichloromethane (300 mL) and acetonitrile (300 mL), $RuCl_3$ (1.16 g, 5 mol%) was added and the mixture was cooled to 10 °C and sodium metaperiodate (60 g) in water (400 mL) was added dropwise. It was stirred at 10 °C for 20 hr. The reaction mixture was diluted with water, The layers were separated and to the organic layer, was added saturated brine solution with stirring followed by 3% sodium sulfide solution drop wise for the decolourisation (dark green to pale yellow). The layers were separated, the organic layer was dried over sodium sulfate and evaporated at reduced pressure to afford pure product. MW calcd for $C_{20}H_{35}BrO_7$ 467.39; Found 465.4 (M-2H). ^{1}H NMR (400 MHz, DMSO) δ 11.94 (s, 2H), 4.88 – 4.69 (m, 1H), 3.53 (t, J = 6.6 Hz, 2H), 2.43 (t, J = 7.2 Hz, 2H), 2.17 (t, J = 7.4 Hz, 4H), 2.09 – 1.95 (m, 2H), 1.90 (s, 3H), 1.46 (s, 7H), 1.23 (s, 15H).

Synthesis of 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioic acid (252)

The Bromoacid **251** (2 mmol) is dissolved in 2M solution of dimethylamine in THF (20 mL) and to it 1 g of anhudrous K_2CO_3 was added and the mixture was heated in a pressure bottle at 50 °C overnight. The TLC showed the completion of the reaction. The reaction mixture was acidified with acetic acid and diluted with water (100 mL) and extracted with dichloromethane (2 x 60 mL). The combined organic layers were concentrated dried and used as such in the next reaction. MW calcd for $C_{23}H_{43}NO_6$ 429.59; Found 430.6 $(MH)^+$. 1H NMR (400 MHz, DMSO) δ 11.87 – 11.82 (m, 7H), 5.75 (d, J = 0.7 Hz, 15H), 4.85 – 4.69 (m, 38H), 3.64 – 3.55 (m, 12H), 3.35 – 2.83 (m, 106H), 3.01 – 2.90 (m, 59H), 2.94 (ddd, J = 30.6, 7.7, 4.0 Hz, 63H), 2.90 – 2.73 (m, 9H), 2.70 (s, 221H), 2.57 – 2.46 (m, 91H), 2.44 – 2.30 (m, 76H), 2.17 (t, J = 7.3 Hz, 147H), 1.89 (tq, J = 15.5, 7.6 Hz, 88H), 1.79 – 1.69 (m, 13H), 1.65 – 1.32 (m, 311H), 1.28 (d, J = 46.0 Hz, 598H).

Synthesis of 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioyl chloride (253)

The diacid **252** is converted to the corresponding diacid chloride **253** by treating it with oxalyl chloride in dichloromethane in the presence of catalytic DMF and the crude acid chloride obtained after the concentration of the reaction mixture was used as such for the coupling with different alcohols.

General procedure for the synthesis of cationic lipids 254a-n

To a solution of the acid chloride **253** (500 mg, 1 mmol) in dichloromethane (30 mL) the corresponding alcohol (5 equivalent) was added at room temperature followed by solid K_2CO_3 (1 g) and the solution was stirred for 16 h at room temperature. The reaction mixture was diluted with dichloromethane (100 mL) and washed with satd. $NaHCO_3$ (100 mL) and the organic layer was dried (Anhyd. Na_2SO_4) and concentrated to obtain the crude product which was purified by Combiflash Rf purification system.

Compound **254b:** By using the above procedure the lipid **254b** was isolated in 72% yield (554 mg). 1H NMR (400 MHz, CDCl3) δ 4.91 – 4.78 (m, 1H), 4.05 (t, J = 6.7 Hz, 4H), 3.81 (s, 6H), 3.63 (t, J = 6.4 Hz, 1H), 2.29 (dt, J = 15.2, 7.5 Hz, 8H), 2.21 (s, 6H), 1.84 – 1.69 (m, 2H), 1.57 (dt, J = 13.4, 5.2 Hz, 9H), 1.53 – 1.40 (m, 4H), 1.27 (s, 43H). 13C NMR (101 MHz, cdcl3) δ 174.45, 174.13, 173.59, 77.54, 77.22, 76.91, 74.34, 64.54, 59.17, 51.65, 45.67, 34.56, 34.35, 34.27, 32.67, 29.59, 29.40, 29.33, 29.31, 29.25, 28.83, 26.06, 25.51, 25.18, 25.11, 23.38. MW calcd for $C_{43}H_{79}NO_{10}$ 770.09; Found 770.68.

Compound **254c:** By using the above procedure the lipid **254c** was isolated in 69% (490 mg). 1H NMR (400 MHz, CDCl3) δ 5.71 – 5.36 (m, 4H), 4.89 – 4.72 (m, 1H), 4.59 (d, J = 6.8 Hz, 4H), 2.26 (ddd, J = 22.3, 13.0, 8.6 Hz, 9H), 2.19 (s, 6H), 2.12 – 1.95 (m, 4H), 1.82 – 1.68 (m, 2H), 1.63 – 1.37 (m, 8H), 1.37 – 1.00 (m, 32H), 0.85 (t, J = 6.8 Hz, 6H). 13C NMR (101 MHz, cdcl3) δ 173.94, 173.57, 135.61, 123.57, 77.54, 77.22, 76.91, 74.34, 60.40, 59.16, 45.65, 34.52, 34.33, 32.66, 31.88, 29.59, 29.57, 29.38, 29.28, 29.06, 27.75, 25.49, 25.14, 23.35, 22.81, 14.28. MW calcd for $C_{43}H_{83}NO_6$: 710.12; Found 710.81.

Compound **254d**: By using the above procedure the lipid **254d** was isolated in 67% yield (456 mg). 1H NMR (400 MHz, CDCl3) δ 4.92 – 4.78 (m, 1H), 4.05 (t, J = 6.7 Hz, 4H), 3.63 (t, J = 6.4 Hz, 1H), 2.39 – 2.24 (m, 8H), 2.21 (s, 6H), 1.89 – 1.70 (m, 2H), 1.69 – 1.54 (m, 8H), 1.51 (dd, J = 17.2, 6.3 Hz, 4H), 1.27 (s, 42H), 0.88 (t, J = 6.8 Hz, 6H). MW calcd for: $C_{41}H_{79}NO_6$: 682.07; Found 682.96.

Compound **254e**: By using the above procedure the lipid **254e** was isolated in 70% (474 mg). 1H NMR (400 MHz, CDCl3) δ 5.49 (ddd, J = 12.9, 9.8, 7.3 Hz, 2H), 5.40 – 5.23 (m, 2H), 4.92 –

4.77 (m, 1H), 4.05 (t, J = 6.9 Hz, 4H), 2.32 (ddd, J = 23.4, 14.5, 7.1 Hz, 12H), 2.21 (s, 6H), 2.07 – 1.91 (m, 4H), 1.84 – 1.70 (m, 2H), 1.66 – 1.39 (m, 8H), 1.40 – 1.15 (m, 26H), 0.88 (t, J = 6.8 Hz, 5H). MW calc. for $C_{41}H_{75}NO_6$ (MH^+): 678.04, found: 678.5.

Compound **254f**: By using the above procedure the lipid **254f** was isolated in 73% (559 mg). 1H NMR (400 MHz, CDCl3) δ 5.87 – 5.62 (m, 2H), 5.55 (dtt, J = 9.1, 6.4, 1.3 Hz, 2H), 4.93 – 4.75 (m, 1H), 4.50 (dd, J = 6.5, 0.6 Hz, 4H), 2.40 – 2.17 (m, 13H), 2.12 – 1.95 (m, 4H), 1.89 – 1.67 (m, 2H), 1.69 – 1.44 (m, 7H), 1.41 – 1.12 (m, 25H), 0.88 (t, J = 6.9 Hz, 5H). MW calc. for $C_{41}H_{75}NO_6$ (MH^+): 678.04, found: 678.5.

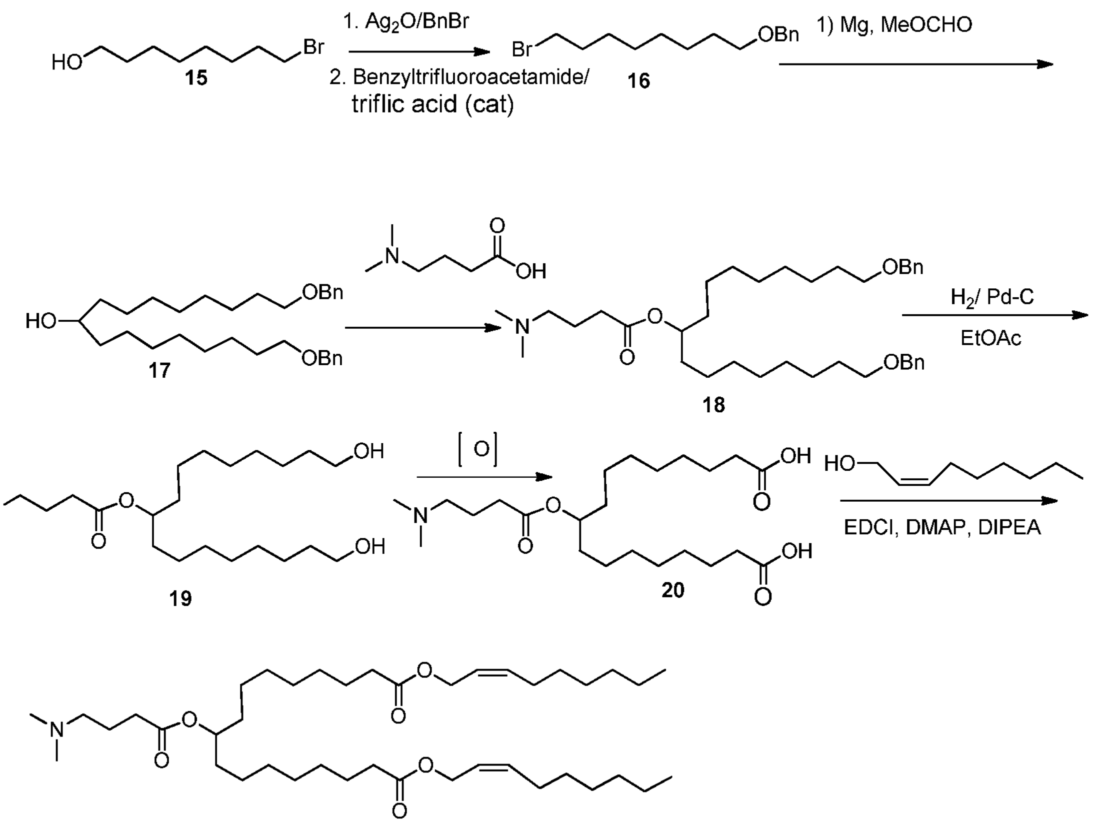
Compound **254 g:** By using the above procedure the lipid **254g** was isolated in 63% (432 mg). 1H NMR (400 MHz, CDCl3) δ 4.93 – 4.77 (m, 1H), 4.20 – 3.95 (m, 4H), 2.44 – 2.23 (m, 8H), 2.21 (s, 6H), 1.84 – 1.66 (m, 3H), 1.68 – 1.34 (m, 15H), 1.35 – 1.17 (m, 20H), 1.17 – 1.04 (m, 5H), 0.88 (dd, J = 12.4, 6.6 Hz, 16H). MW calcd for $C_{43}H_{83}NO_6$: 710.12; Found 710.81.

Compound **254h:** By using the above procedure the lipid **254h** was isolated in 66% (466 mg). 1H NMR (400 MHz, CDCl3) δ 5.08 (ddd, J = 7.1, 5.9, 1.3 Hz, 2H), 4.91 – 4.75 (m, 1H), 4.22 – 3.97 (m, 4H), 2.39 – 2.22 (m, 8H), 2.23 (d, J = 16.7 Hz, 7H), 2.09 – 1.84 (m, 4H), 1.86 – 1.71 (m, 3H), 1.71 – 1.02 (m, 44H), 0.91 (t, J = 4.9 Hz, 6H). MW calcd for $C_{43}H_{79}NO_6$: 706.12; Found 706.81.

Example 19

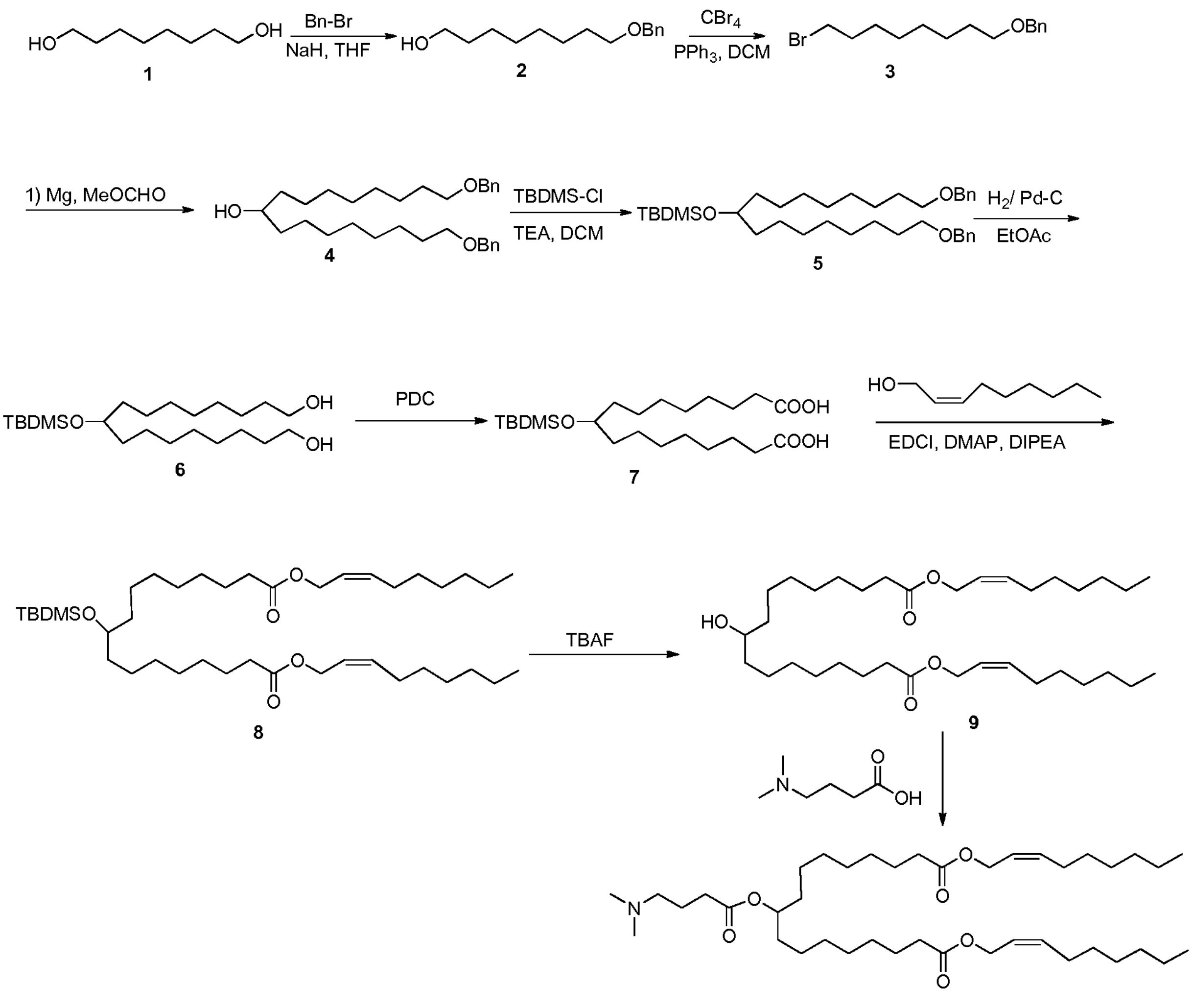
In another approach the following synthetic approach is used for the synthesis of Compound 1 of the present invention.

Scheme 19



Example 20

Scheme 20



8-benzyloxy-octan-1-ol (2): To a stirred suspension of NaH (60% in oil, 82 g, 1.7096 mol) in 500mL anhydrous DMF, a solution of compound **1** (250g, 1.7096 mol) in 1.5 L DMF was added slowly with dropping funnel at 0° C. Reaction mixture was stirred for 30 min and to it Benzyl bromide (208.86 mL, 1.7096 mol) was added slowly under nitrogen atmosphere. Reaction was then warmed to ambient temperature and stirred for 10 h. After completion of reaction, mixture was quenched with crushed ice (~2kg) and extracted with Ethyl acetate (2 x 1L). Organic layer washed with water (1L) to remove unwanted DMF, dried over Na_2SO_4 and evaporated to dryness under vacuum. The crude compound was purified on 60-120 silica gel, eluted with 0-5% MeOH in DCM to afford compound **2** (220g, 54%) as pale yellow liquid. H^1 NMR (400MHz, $CDCl_3$): δ = 7.33-7.24 (m, 5H), 4.49 (s, 2H), 3.63-3.60 (m, 2H), 3.47-3.43 (m, 2H), 1.63-1.51 (m, 4H), 1.39-1.23 (m, 8H).

(8-bromo-octyloxymethyl)-benzene (3): Compound **2** (133g, 0.5635mol) was dissolved in 1.5 L of DCM, CBr_4 (280.35g, 0.8456mol) was added into this stirring solution and reaction mixture was cooled to 0°C under inert atmosphere. PPh_3 (251.03g, 0.9571mol) was then added in portions keeping the temperature below 20°C and after complete addition reaction was stirred for 3 h at room temperature and monitored by TLC. After completion of reaction, solid (PPh_3O) precipitated out from the reaction mixture was filtered off and filtrate was diluted with crushed ice (~ 1.5kg) and extracted with DCM (3 x 750mL). Organic layer was separated, dried over an. Na_2SO_4 and distilled under vacuum. Resulting crude compound was chromatographed on 60-120 mesh silica gel column using 0-5 % ethyl acetate in hexanes as eluting system to give compound **3** (150g, 89%) as pale yellow liquid. H^1 NMR (400MHz, $CDCl_3$): δ = 7.33-7.25 (m, 5H), 4.49 (s, 2H), 3.47-3.41 (m, 2H), 3.41-3.37 (m, 2H), 1.86-1.80 (m, 4H), 1.62-1.56(m, 2H), 1.42-1.29 (m, 8H).

1, 17-bis-benzyloxy-heptadecan-9-ol (4): To freshly activated Mg turnings (24.08g, 1.003mol) was added 200mL anhydrous THF was added followed by the addition of pinch of iodine into the mixture under inert atmosphere. After initiation of the Grignard formation a solution of Compound **3** (150g, 0.5016mol) in 1 L of dry THF was added slowly controlling the exothermic reaction. After complete addition reaction was refluxed for 1h and then cooled to room temperature. (60.24g, 1.0033mol) methyl formate was then added slowly and reaction was continued for 2h. After completion, the reaction was quenched by slow addition of 10% HCl followed by water (1 L) and extracted with Ethyl Acetate (3 x 1L). Organic layer was taken in 5 lit beaker, diluted with 500mL of methanol and cooled to 0°C. To this solution excess of $NaBH_4$ (~ 5eq) was added in portions to ensure the hydrolysis of formate ester which was not cleaved by addition of HCl. Resulting solution was stirred for an hour and then volatilites were stripped off under vacuum. Residue was taken in water (1 L) and acidified by 10% HCl solution (P^H 4).

Product was then extracted out with ethyl acetate (3 x 1 L). Organic phase was then dried and concentrated on rotary evaporator to get the desired compound **4** (57g, 24%) as solid. H^1 NMR (400MHz, $CDCl_3$): δ = 7.35-7.32 (m, 8H), 7.29-7.24 (m, 2H), 4.49 (s, 4H), 3.56 (m, 1H), 3.46-3.43 (m, 4H), 1.63-1.56 (m, 4H), 1.44-1.34 (m, 28H). C^{13} NMR (100MHz, $CDCl_3$): δ = 138.56, 128.21, 127.49, 127.34, 72.72, 71.76, 70.37, 37.37, 29.64, 29.56, 29.47, 29.33, 26.07, 25.54.

[9-benzyloxy-1-(8-benzylozy-octyl)-nonyloxy]-*tert*-butyl-dimethyl-silane (5): Compound **4** (56 g, 0.1196 mol) was dissolved in 700 mL of anhydrous THF and cooled to 0 °C. TBMS-Cl (36.06g, 0.2396mol) was added slowly followed by addition of Imidazole (32.55 g, 0.4786 mol) under inert atmosphere. Reaction was then stirred at room temperature for 18h. Reaction was judged complete by TLC and then quenched with ice (~1kg) and extracted with Ethyl acetate (3 x 500mL). Organic layer was separated, washed with Sat $NaHCO_3$ solution to remove the acidic impurity, dried over Na_2SO_4 and evaporated under reduce pressure to obtain crude compound which was purified by silica gel (60-120 mesh) and eluted with 0- 10% ethyl acetate hexane to yield (60g, 82%) of compound **5** as yellowish oil. H^1 NMR (400MHz, $CDCl_3$): δ = 7.33-7.24 (m, 10H), 4.49 (s, 4H), 3.60-3.57 (m, 1H), 3.46-3.43 (m, 4H), 1.61-1.54 (m, 4H), 1.41-1.26 (m, 28H), 0.87 (s, 9H), 0.02 (s, 6H)

9-(*tert*-butyl-dimethyl-silanyloxy)-heptadecane-1, 17-diol (6): Compound **5** (60 g, 0.1030 mol) was dissolved in 500mL ethyl acetate and degassed with N_2 for 20 min. (10 wt %) Pd on carbon (12 g) was added and reaction was stirred under H_2 atmosphere for 18 h. After completion of reaction (by TLC) mixture was filtered through celite bed and washed with ethyl acetate. Filtrate was evaporated under vacuum. The compound **6** (19 g, 46%) thus obtained was pure enough to carry out the next reaction. H^1 NMR (400MHz, $CDCl_3$): δ = 3.64-3.58 (m, 5H), 1.59 (br, 2H), 1.57-1.51 (m, 4H), 1.38-1.22 (m, 28H), 0.87 (s, 9H), 0.02 (s, 6H).

9-(*tert*-butyl-dimethyl-silanyloxy)-heptadecanedioic acid (7): To a stirred solution of **6** (2 g, 0.0049 mol) in anhydrous DMF (40 mL) was added pyridinium dirchromate (2.7 g, 0.0074 mol) at 0 °C under inert atmosphere. Reaction mixture was then allowed to warm to room temperature over a period of 10-15 minutes and continued for 24h. Progress of the reaction was monitored by TLC. After complete oxidation reaction was diluted with water (100mL). Aqueous phase was extracted with DCM (3 x 40mL). Organic phase was washed with brine (1x 25mL) and concentrated under vacuum to afford crude acid which was then purified by (100-200 mesh) silica gel column using 0-30% ethyl acetate in hexanes system. Pure product **26-003** was obtained (0.7g, 33%) as pale yellow oil. H^1 NMR (400MHz, $CDCl_3$): δ = 3.61-3.56 (m, 1H), 2.35-2.32 (m, 4H), 1.64-1.59 (m, 4H), 1.40-1.19 (m, 24H), 0.86 (s, 9H), 0.017 (s, 6H); LC-MS [M+H] - 431.00; HPLC (ELSD) purity – 96.94%

Di((Z)-non-2-en-1-yl) 9-((tert-butyldimethylsilyl)oxy)heptadecanedioate (8): The diacid **7** (0.42 g, 0.97 mmol) was dissolved in 20 mL of dichloromethane and to it *cis*-2-nonen-1-ol (0.35 g, 2.44 mmol) was added followed by Hunig's base (0.68 g, 4.9 mmol) and DMAP (12 mg). To this mixture EDCI (0.47 g, 2.44 mmol) was added and the reaction mixture was stirred at room temperature overnight and the TLC (silica gel, 5% MeOH in CH_2Cl_2) showed complete disappearance of the starting acid. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated $NaHCO_3$ (50 mL), water (60 mL) and brine (60 mL). The combined organic layers were dried over anhyd. Na_2SO_4 and solvents were removed *in vacuo*. The crude product thus obtained was purified by Combiflash Rf purification system (40 g silicagel, 0-10 % MeOH in CH_2Cl_2) to isolate the pure product **8** (0.35 g, 53%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, *J* = 10.9, 7.4 Hz, 2H), 5.58 – 5.43 (m, 2H), 4.61 (d, *J* = 6.8 Hz, 4H), 3.71 – 3.48 (m, 1H), 2.30 (t, *J* = 7.6 Hz, 4H), 2.20 – 1.98 (m, 4H), 1.71 – 1.53 (m, 4H), 1.31 (ddd, *J* = 8.3, 7.0, 3.7 Hz, 34H), 1.07 – 0.68 (m, 14H), 0.02 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.18, 139.81, 127.78, 81.73, 81.42, 81.10, 76.72, 64.59, 41.52, 41.32, 38.76, 36.09, 34.10, 33.93, 33.80, 33.70, 33.59, 33.55, 33.26, 31.95, 30.34, 29.69, 29.58, 29.39, 27.01, 22.56, 18.48, 0.01.

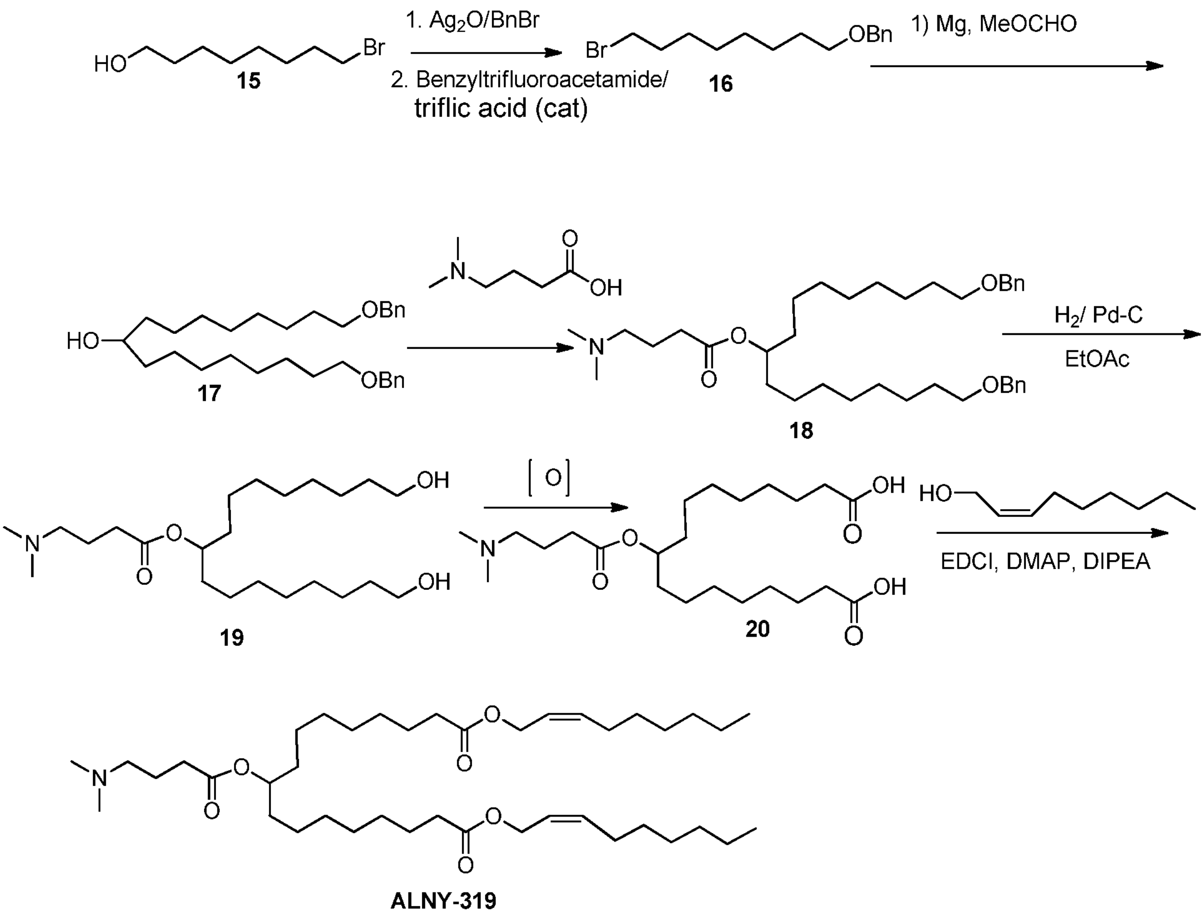
Di((Z)-non-2-en-1-yl) 9-hydroxyheptadecanedioate (9): The silyl protected diester **8** (0.3 g, 0.44 mmol) was dissolved in 1 M solution of TBAF in THF (6 mL) and the solution was kept at 40 °C for two days after which the TLC showed the completion of the reaction. The reaction mixture was diluted with water (60 mL) and extracted with ether (2 x 50 mL). The combined organic layers were concentrated and the thus obtained crude product was purified by column to isolate the pure product (0.097 g, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dt, *J* = 10.9, 7.4 Hz, 2H), 5.52 (dt, *J* = 11.0, 6.8 Hz, 2H), 4.61 (d, *J* = 6.8 Hz, 4H), 3.57 (s, 1H), 2.30 (t, *J* = 7.5 Hz, 4H), 2.09 (q, *J* = 7.1 Hz, 4H), 1.75 – 1.53 (m, 4H), 1.53 – 1.06 (m, 36H), 0.88 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.98, 135.64, 123.57, 77.54, 77.22, 76.91, 72.14, 60.41, 37.69, 34.54, 31.89, 29.70, 29.60, 29.44, 29.29, 29.07, 27.76, 25.80, 25.15, 22.82, 14.29.

Di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate: The alcohol **9** (0.083 g, 0.147 mmol) was dissolved in 20 mL of dichloromethane and to it dimethylaminobutyric acid hydrochloride (0.030 g, 0.176 mmol) was added followed by Hunig's base (0.045 g, 0.44 mmol) and DMAP (2 mg). To this mixture EDCI (0.034 g, 0.176 mmol) was added and the reaction mixture was stirred at room temperature overnight and the TLC (silica gel, 10% MeOH in CH_2Cl_2) showed complete disappearance of the starting alcohol. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated $NaHCO_3$ (50 mL), water (60 mL) and brine (60 mL). The combined organic layers were dried over anhyd. Na_2SO_4 and solvents were removed *in vacuo*. The crude product thus obtained was purified by Combiflash Rf purification system (40 g silicagel, 0-10 % MeOH in CH_2Cl_2) to isolate the pure product (0.062 g, 62%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 – 5.58 (m, 2H), 5.51 (dtt, *J* = 9.7, 6.8, 1.3 Hz, 2H), 4.95 – 4.75 (m, 1H), 4.61 (d, *J* = 6.8 Hz, 4H), 2.35 – 2.24 (m, 8H), 2.22 (d, *J* = 7.9 Hz, 6H), 2.09 (q, *J* = 6.9 Hz, 4H), 1.83 – 1.72 (m, 2H), 1.60 (dd, *J* = 14.4, 7.2 Hz, 4H), 1.49 (d, *J* = 5.7 Hz, 4H), 1.41 – 1.13 (m, 30H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.72, 173.36, 135.40, 123.35, 74.12, 60.18, 58.95, 45.46, 34.30, 34.11, 32.45, 31.67, 29.38, 29.35, 29.17, 29.07, 28.84, 27.53, 25.28, 24.93, 23.16, 22.59, 14.06. MW calc. for $C_{41}H_{75}NO_6$ (MH^+): 678.04, found: 678.5.

In another embodiment the following shorter route was used for the synthesis of the **di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate.** The commercial 9-bromonon-1-ene **10** was treated with magnesium to form the corresponding Grignard reagent which was reacted with ethylformate to give the corresponding adduct **11** which on treatment with bromobutyryl chloride to provide the bromoester **12**. The bromoester **12** ontreatment with RuO_4 provided the diacid **13**. The bromodiacid **13** on treatment with dimethylamine provided the amino diacid **14.** The aminodiacid **14** on coupling with the alcohol **15** provided the product in good yields.

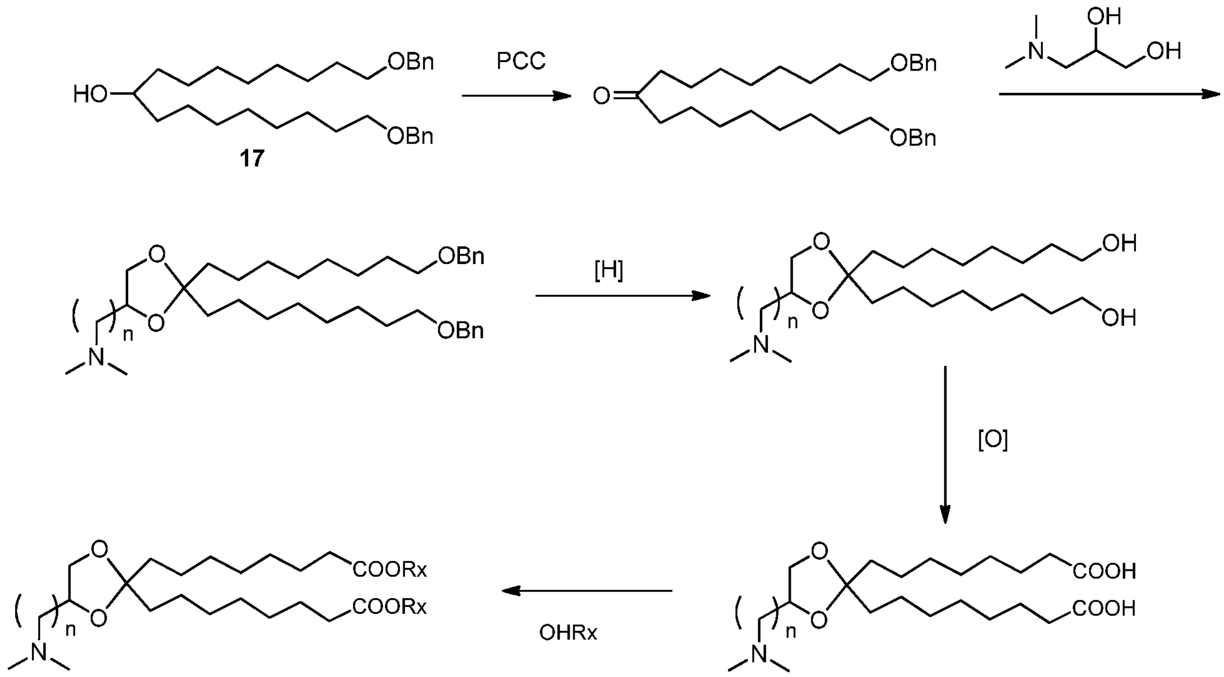
Example 21

Scheme 21



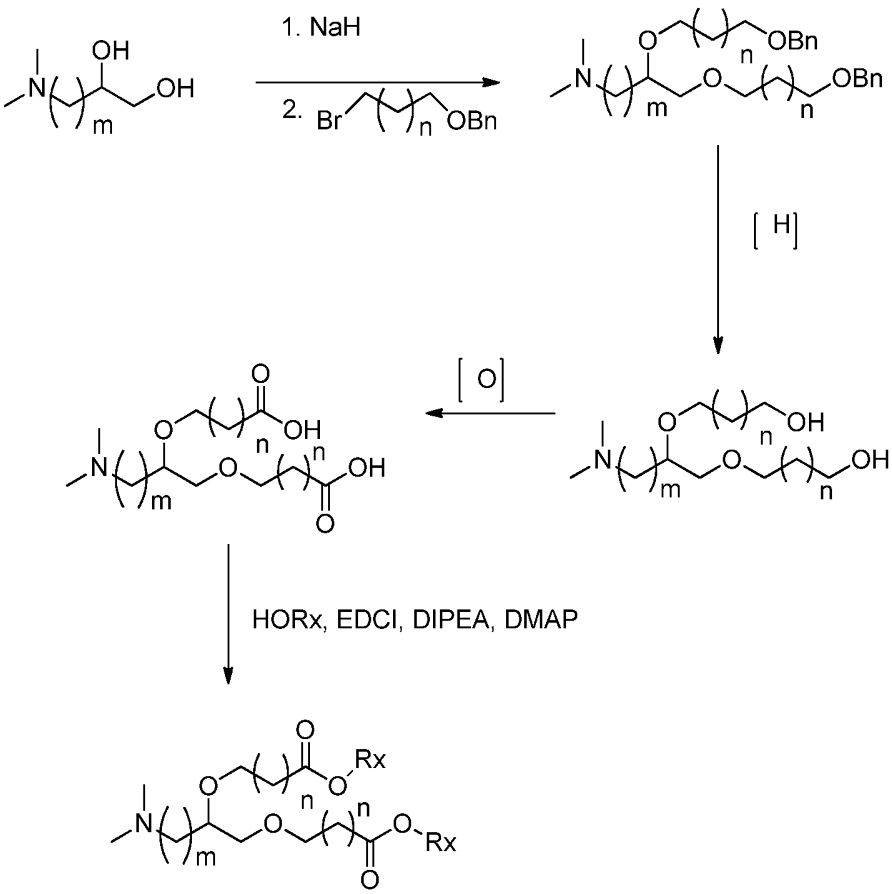
Example 22

Scheme 22



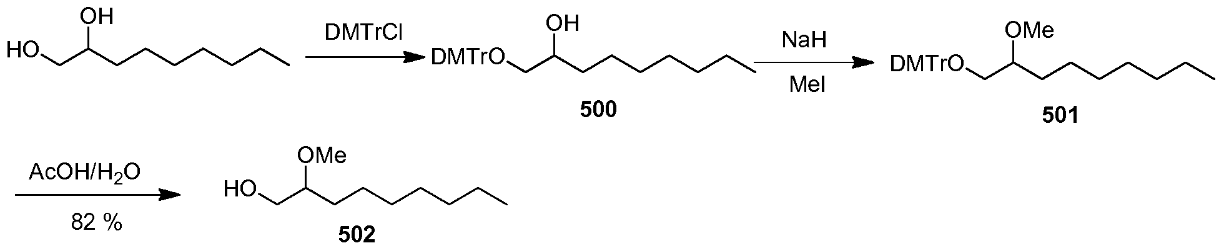
Example 23

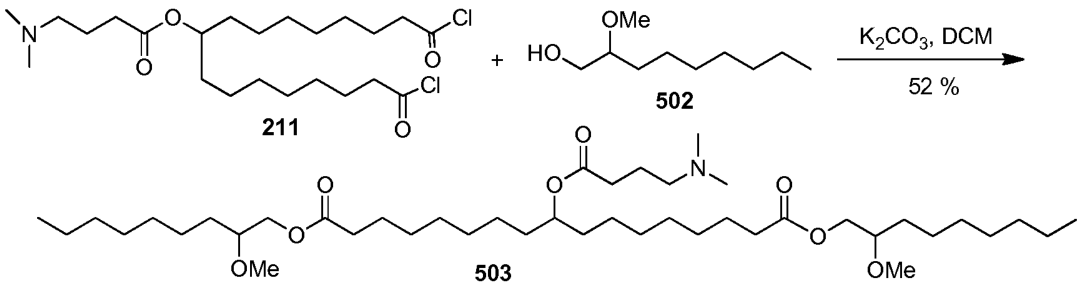
Scheme 23



Example 24

Scheme 24





Compound **501**: To a stirred solution of 2-hydroxy 1-octanol 5g (31.25 mmol), DMAP 0.38g (3.1 mmol) in dry pyridine (100 mL) was added DMTr-Cl and stirred at room temperature for 14 h. 10 mL of water was added and extracted with ethyl acetate, washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentration of the solvent gave 20g of crude product **500** which was co-evaporated with toluene twice and used for the next step without further purification. To the above crude DMTr ether in dry THF (250 mL) were added NaH and iodo methane at $0^{\circ}C$ and then brought to room temperature over 30 min. and then stirred for two days. 5 mL of water was added and concentrated followed by column chromatography (0-30 % ethyl acetate in hexane) gave the corresponding product **501** (10.25 g, R_f: 0.45, 20 % ethyl acetate in hexane) and 8.4 g of recovered starting material **500**. ^1H NMR (400 MHz, $CDCl_3$) δ 7.47-6.8 (m, 13 H), 3.79 (s, 6 H), 3.42 (s, 3 H), 3.29-3.26 (m, 1H), 3.13-3.04 (m, 2 H), 1.55-1.47 (m, 2 H), 1.3-1.2 (m, 10 H), 0.89 (t, J = 6.4 Hz, 3 H).

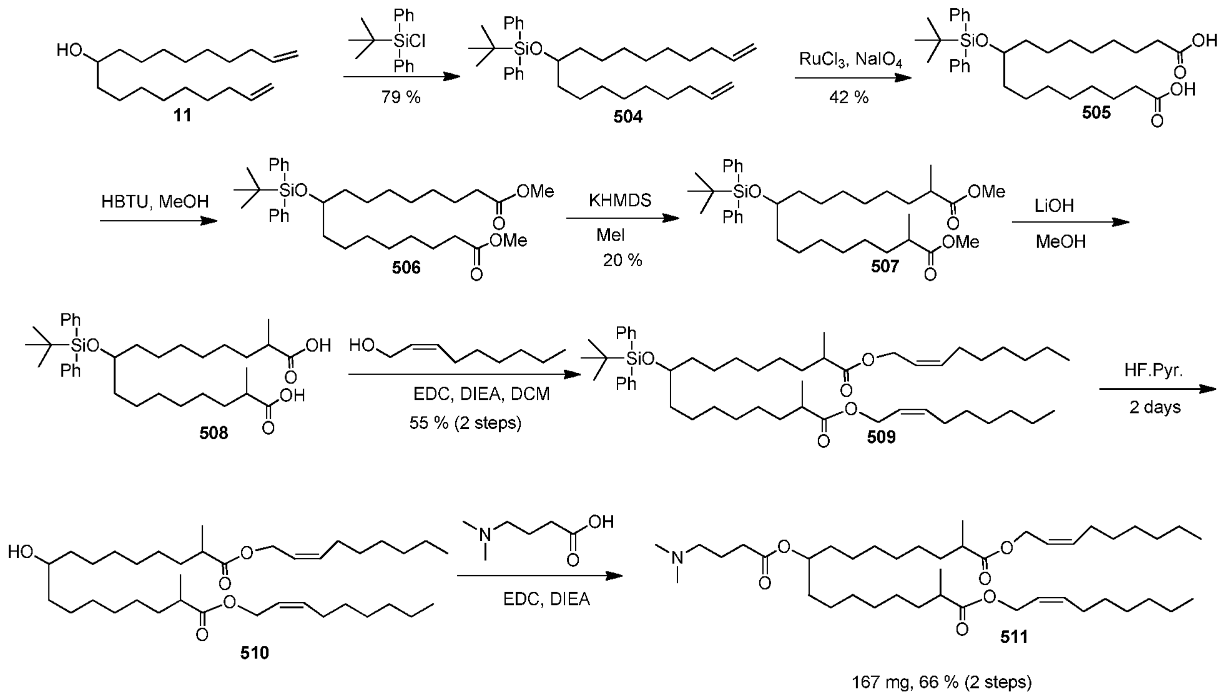
Alcohol **502**: The compound **501** (10.25 g, 21.5 mmol) was dissolved in 75 mL of 80 % acetic acid and stirred at room temperature for 14 h. 10 mL of methanol was added and concentrated, followed by column chromatography (0-50 % ethyl acetate in hexane) yielded the expected product **502** as colorless oil (1.8 g, 82 %, R_f: 0.3, 30 % ethyl acetate in hexane). ^1H NMR (400

MHz, $CDCl_3$) δ 3.71-3.65 (m, 1 H), 3.5-3.45 (m, 1 H), 3.41 (s, 3 H), 3.28-3.25 (m, 1 H), 1.93-1.9 (m, 1 H), 1.45-1.41 (m, 2 H), 1.39-1.27 (m, 10 H), 0.88 (s, J = 6.8 Hz, 3 H).

Compound **503**: Compound **503** was synthesized following general experimental procedure for compound **213**. 0.3g as pale yellow oil (52 %, R_f = 0.2, 5 % methanol in dichloromethane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87-4.84 (m, 1 H), 4.18-4.00 (m, 4 H), 3.4 (s, 6 H), 3.37-3.19 (m, 2 H), 2.34-2.26 (m, 6 H), 2.2 (s, 6 H), 1.8-1.6 (m, 2 H), 1.63-1.2 (m, 50 H), 0.88 (s, J = 6.8 Hz, 6 H).

Example 25

Scheme 25



Compound **504**: To a stirred solution of alcohol **11** (4.01 g, 22.25 mmol), TBDPS-Cl (12.24 g, 44.5 mmol) and DMAP (0.54 g, 4.42 mmol) was added triethyl amine (8.99 g, 90 mmol) and stirred at room temper for 14 h. To the above solution was added imidazole (1.51 g, 22.25 mmol) and continued to stir for 14 h at room temperature. 20 mL of water was added and extracted with DCM followed by washing with 2N HCl, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-10 % ethyl acetate in hexane) to yield compound **504** (7.38 g, 79 %, R_f: 0.8, 5 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.66 (m, 4 H), 7.43-7.33 (m, 6 H), 5.86-5.76 (m, 2 H), 5.02-4.91 (m, 4 H), 3.73-3.67 (m, 1 H), 2.04-1.99 (m, 4H), 1.42-1.08 (m, 24 H), 1.05 (s, 9 H).

Compound **505**: To a stirred solution of diene **504** (7.38 g, 17.6 mmol) and $RuCl_3$ (0.18g, 0.88 mmol) in 400 mL of DCM/CH_3CN(1:1) was added $NaIO_4$ (37.6 g, 176 mmol) dissolved in 400 mL of water drop wise around 5° C over 30 min. and stirred at room temperature for 3 h. The organic layer was separated followed by washing with 3 % Na_2S solution (100 mL), water (250 mL) brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product **505** (4 g, 42 %, R_f: 0.3, 40 % ethyl acetate in hexane), which was used for the next step without further purification.

Compound **506**: To a stirred solution of the acid **505** (4 g, 7.22 mmol), HBTU (6.02 g, 15.88 mmol), HOBt (2.14 g, 15.88 mmol) and DMAP (88 mg, 0.72 mmol) in 75 mL of dry DCM was added 5 mL of methanol and stirred at room temperature for 14 h. 10 mL of water was added followed by extraction with DCM (3 x 50 mL), washing with saturated $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-30 % ethyl acetate in hexane) to yield compound **506** (2 g, 47.6 %, R_f: 0.3, 10 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67-7.65 (m, 4 H), 7.41-7.33 (m, 6 H), 3.70-3.64 (m, 1 H), 3.66 (s, 6 H), 2.28 (t, J = 7.2 Hz, 4 H), 1.63-1.07 (m, 24H), 1.04 (s, 9 H).

Compound **507**: To a stirred solution of dimethyl ester **506** (1.0 g, 1.79 mmol) in dry THF (20 mL) were added KHMDS (0.752 g, 3.76 mmol) and methyl iodide (0.762 g, 5.37 mmol) at 0°C and then brought to room temperature over 30 min. and stirred for 24 h. 10 mL of sat. NH_4Cl solution was added followed by extraction with ethyl acetate (3 x 50 mL), washing with water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product, which was purified by column chromatography (0-5 % ethyl acetate in hexane) to obtain the product **507** (0.218g, 20 %, R_f: 0.8, 5 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.68-7.65 (m, 4 H), 7.41-7.33 (m, 6 H), 3.70-3.67 (m, 1 H), 3.67 (s, 6 H), 2.43-2.38 (m, 2 H), 1.59-1.07 (m, 24 H), 1.13 (d, J = 7.2 Hz, 6 H), 1.04 (s, 9 H).

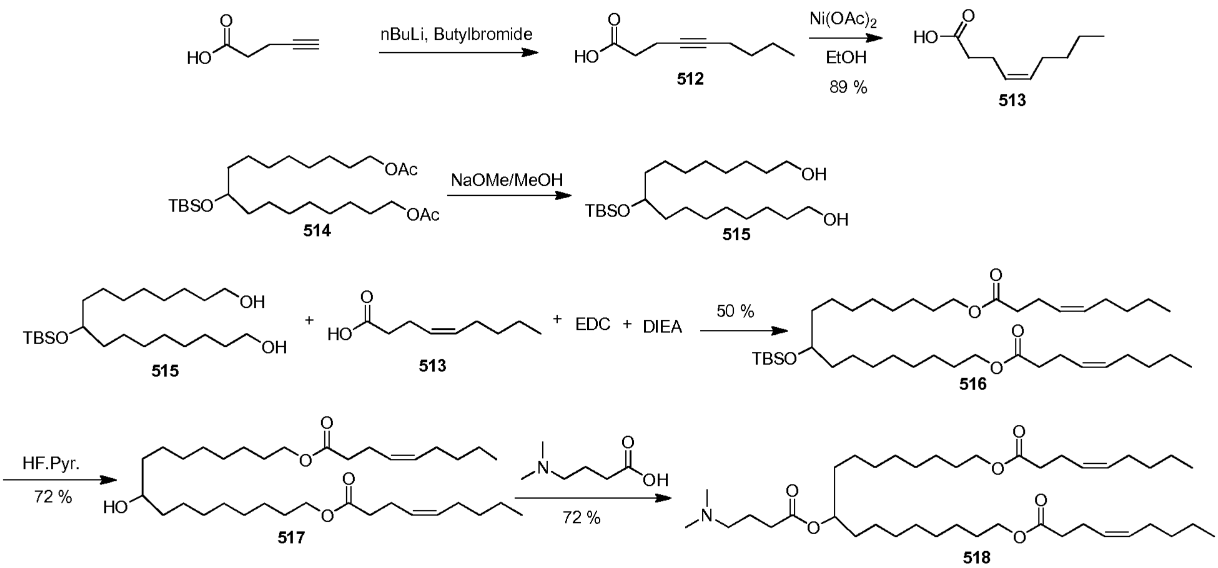
Compound **509**: To a stirred solution of methyl ester **507** (0.4 g, 0.66 mmol) in 10 mL of MeOH/THF (1:1) was added LiOH (0.079 g, 3.27 mmol) in 1 mL of water and stirred at room temperature for 24 h. To the above solution was added KOH (0.183 g, 3.27 mmol) in 1 mL of water and stirred for another 2 days. 2 mL of sat. NH_4Cl solution was added followed by extraction with ethyl acetate (3 x 25 mL), washing with water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product **508** (0.45 g, R_f: 0.2, 10 % ethyl acetate in hexane), which was used for the next step without further purification. To a stirred solution of the above di-acid **508** (0.45 g), *cis*-2-Nonen-1-ol (0.66 g, 4.6 mmol) and EDC•HCl (0.82 g, 4.6 mmol) in dry DCM (15 mL) was added DIEA (1.2 g, 9.24 mmol) and stirred at room temperature for 3 days. 10 mL of water was added followed by extraction with DCM followed by washing with 2N HCl, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-10 % ethyl acetate in hexane) to yield compound **509** (0.3 g, 55 %, R_f: 0.5, 3 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67-7.65 (m, 4 H), 7.42-7.33 (m, 6 H), 5.67-5.6 (m, 2 H), 5.55-5.49 (m, 2 H), 4.615 (d, J = 4 Hz, 4 H), 3.71-3.65 (m, 1 H), 2.44-2.35 (m, 2 H), 2.10 (q, J = 8.0 Hz, 4 H), 1.64-1.07 (m, 40 H), 1.13 (d, J = 8.0 Hz, 6 H), 1.04 (s, 9 H), 0.86 (t, J = 10 Hz, 6 H).

Compound **511**: To a stirred solution of silyl ether **509** (0.3 g, 0.36 mmol) in dry THF were added pyridine (1 mL) and HF•Pyr., (1 mL) drop wise and stirred at 45°C for 48 h. The solvent was evaporated and used for the next step without purification.

To a stirred solution of the above crude alcohol **510**, *N,N*-Dimethyl amino butyric acid (0.34 g, 2.04 mmol), EDC•HCl (0.39 g, 2.04 mmol) and DMAP (0.06 g, 0.51 mmol) in dry DCM (10 mL) was added DIEA (0.5 g, 3.88 mmol) and stirred at room temperature for 2 days. 10 mL of water was added followed by extraction with DCM (3 x 25 mL), washing with saturated $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-30 % ethyl acetate in 1 % TEA containing hexane) to yield compound **511** (0.167 g, 66 %, R_f: 0.4, 10 % MeOH in DCM). Molecular weight for $C_{43}H_{79}NO_6$ $(M+H)^+$ Calc. 706.59, Found 706.5.

Example 26

Scheme 26



Compound **512:** To a stirred solution of 4-Pentynoic acid in 100 mL of THF/HMPA (4:1) at -78°C was added nBuLi (3.1 g, 49 mmol) drop wise and stirred for 30 min. Then the reaction mixture was brought to 0°C and stirred for 2 h. Again, the reaction mixture was cooled to -78°C and n-butyl bromide (3.07 g, 22.44 mmol) was added drop wise and stirred at room temperature for 14 h. 10 mL of sat. NH_4Cl solution was added followed by extraction with ethyl acetate (3 x 25 mL), washing with water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product , which was purified by column chromatography (0-30 % ethyl acetate in hexane) to yield compound **512** (0.4 g, R_f: 0.8, 30 % ethyl acetate in hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 2.59-2.55 (m, 2 H), 2.49-2.44 (m, 2 H), 2.16-2.11 (m, 2 H), 1.49-1.34 (m, 4 H), 0.9 (t, J = 6.0 Hz, 3 H).

Compound **513:** To a suspension of $Ni(OAc)_2$ (0.45 g, 2.53 mmol) in EtOH (20 mL) was added $NaBH_4$ (0.096 g, 12.65 mmol) portion wise at room temperature and stirred for 15 min. under H_2 atm. Filtered off the solid followed by concentration of the solvent gave compound **513** (0.35 g, 88.6 %, R_f: 0.6, 20 % ethyl acetate in hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 10.88 (br s, 1H), 5.47-5.41 (m, 1H), 5.35-5.31 (m, 1H), 2.43-2.33 (m, 4 H), 2.07-2.03 (m, 2 H), 1.36-2.27 (m, 4 H), 0.9 (t, J = 8.0 Hz, 3 H).

Compound **515:** To a stirred solution of di-acetate **514** (1.5 g, 3.09 mmol) in MeOH (100 mL) was added a piece of sodium metal (0.05 g, 2.17 mmol) and stirred at room temperature for 14 h. Neutralized with dry ice and concentrated followed by extraction with ethyl acetate (3 x 50 mL), washing with water, dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product **515** (1.1 g, 88.7 %) , which was used for the next step without purification.

Compound **516:** To a stirred solution of the above diol **515** (0.4 g, 1 mmol), **513** (0.341 g, 2.19 mmol), DMAP (0.1 g, 0.82 mmol) and EDC•HCl (0.57 g, 2.98 mmol) in dry DCM (15 mL) was added DIEA (5.97 g, 6 mmol) and stirred at room temperature for 2 days. 10 mL of water was added followed by extraction with ethyl acetate followed by washing with 1N HCl, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-10 % ethyl acetate in hexane) to yield compound **516** (0.335 g, 50 %, R_f: 0.6, 5 % ethyl acetate in hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45-5.38 (m, 2H), 5.36-5.29 (m, 2H), 4.06 (t, J = 8 Hz, 4 H), 3.63-3.58 9m, 1 H), 2.39-2.31 (m, 8 H), 2.07-2.02 (m, 4 H), 1.65-1.57 (m, 4 H), 1.4-1.28 (m, 32 H), 0.9 (t, J = 6.0 Hz, 6 H), 0.88 (s, 9 H), 0.03 (s, 6 H).

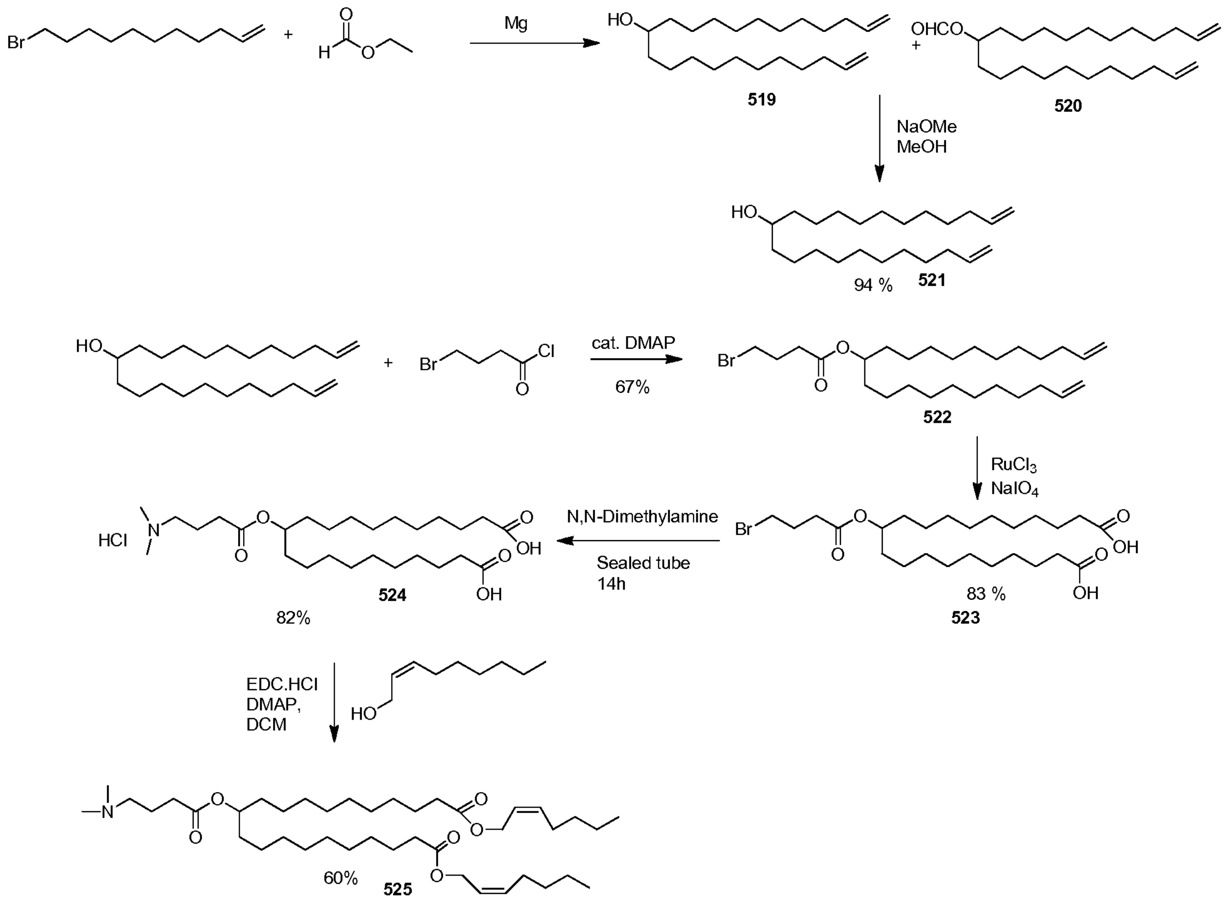
Compound **517:** To a stirred solution of silyl ether **516** (0.3 g, 0.36 mmol) in dry THF (5 mL) were added pyridine (1 mL) and HF•Pyr. (1 mL) drop wise and stirred at 45°C for 24 h. The solvent was evaporated followed by purification by column chromatography gave product **517** (0.2 g, 72 %, R_f: 0.4, 10 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43-5.36 (m, 2H), 5.34-5.27 (m, 2H), 4.04 (t, J = 8 Hz, 4 H), 3.59-3.53 (m, 1 H), 2.37-2.3 (m, 8 H), 2.05-2.0 (m, 4 H), 1.61-1.29 (m, 37 H), 0.88 (t, J = 8.0 Hz, 6 H).

Compound **518:** To a stirred solution of the alcohol **517** (0.2 g, 0.355 mmol), N,N-Dimethyl amino butyric acid (0.36 g, 2.14 mmol), EDC•HCl (0.406 g, 2.14 mmol) and DMAP (0.043 g, 0.36 mmol) in dry DCM (10 mL) was added DIEA (0.55 g, 4.26 mmol) and stirred at room temperature for 2 days. 10 mL of water was added followed by extraction with DCM (3 x 25 mL), washing with saturated $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-30 % ethyl acetate in 1 % TEA containg hexane) to yield compound **518**

(0.172 g, 72 %, R_f: 0.2, 5 % MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43-5.36 (m, 2H), 5.32-5.27 (m, 2H), 4.87-4.83 (m, 1 H), 4.03 (t, J = 6 Hz, 4 H), 2.36-2.2 (m, 6 H), 2.32 (s, 6 H), 2.03-1.25 (m, 40 H), 0.88 (t, J = 6.0 Hz, 6 H).

Example 27

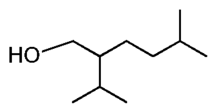
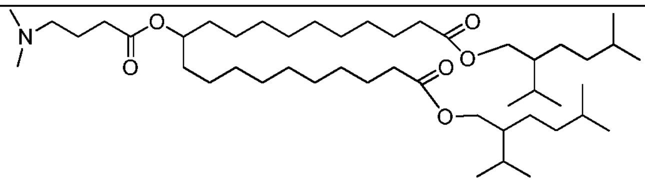
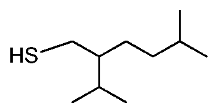
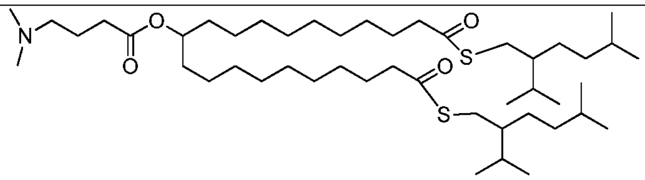
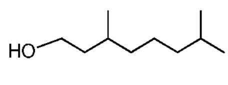
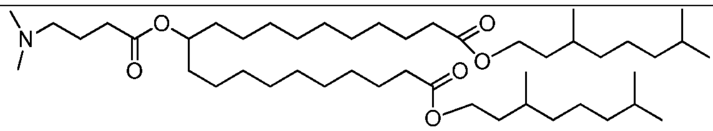
Scheme 27

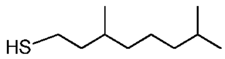
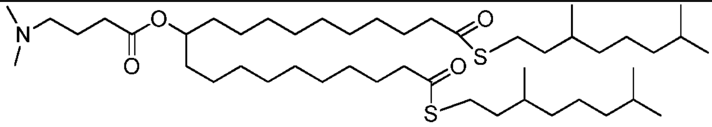
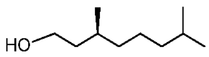
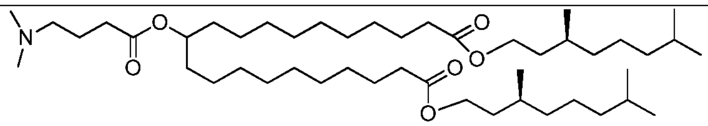
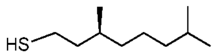
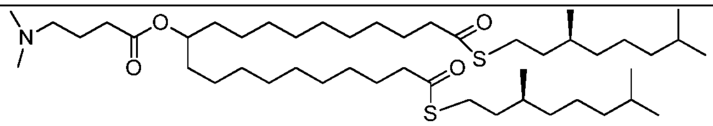


Compound **521:** To a suspension of Mg in Et_2O was added alkyl bromide (25 g, 107.7 mmol) drop wise at 40° C over one hour. Ethyl formate was added to the above reaction mixture at 0-5° C and then the reaction mixture was stirred at room temperature for 14 h. The reaction mixture was poured onto the ice cold sat. NH_4Cl solution followed by extraction with Et_2O (3 x 250 mL), washing with water, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product, which was re-dissoled in MeOH (250 mL) and a small piece of sodium (0.1 g) was added and stirred at room temperature for 14 h. The solvent was evaporated and 100 mL of water was added followed by filtration of the solid, washing with water (2 x 100 mL) gave pale yellow powder **521** (17 g, 94 %, %, R_f: 0.8, 10 % ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84-5.74 (m, 2H), 5.0-4.89 (m, 4H), 3.64-3.49 (m, 1 H), 2.04-1.99 (m, 4 H), 1.79 (br s, 1 H), 1.44-1.23 (m, 32 H).

Compound **522:** To a stirred solution of **521** (10 g, 29.73 mmol) and DMAP (0.1 g, 0.82 mmol) in dry DCM (50 mL) was added 4-bromo butyryl chloride (6.56 g, 35.68 mmol) and stirred at room temperature for 14 h. 5 mL of saturated $NaHCO_3$ was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product which was purified by column chromatography (0-10 % ethyl acetate in hexane) to yield compound **522** (9.6 g, 66.7 %, R_f: 0.9, 5 % ethyl acetate in hexane).

Compound **524:** Oxidation was carried out to get compound **523** (8.6 g, 83.5 %, R_f: 0.1, 5 % MeOH in DCM) following same experimental procedure as for compound **505**. This crude material was dissolved in 2N *N*,*N*-dimethyl amine in THF (20 mL) and heated to 60° C in a sealed tube for 14 h. Concentrated the reaction mixture and then pH of the reaction mixture was brought to 3. This mixture was freeze-dried to obtain compound **524** as HCl salt (4 g, 82 %). Molecular weight for C27H51NO6 $(M+H)^+$ Calc. 486.37, Found 486.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94-4.89 (m, 1 H), 3.32-3.3 (m, 2 H), 3.2-3.16 (m, 2 H), 2.91 (s, 6 H), 2.47 (t, J = 8 Hz, 2 H), 2.28 (t, J = 8 Hz, 4 H), 2.05-1.97 (m, 2 H), 1.61-1.56 (8 H), 1.4-1.25 (m, 22 H).

526		
526s		
527		

527s		
528		
528s		

Synthesis of ester **525, 526, 527 and 528:**

The title compounds were synthesized following the experimental procedure as for compound **516**.

Compound **525:** (0.75 g, 60 %, R_f: 0.3, 5 % MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.65-5.59 (m, 2 H), 5.53-5.47 (m, 2 H), 4.87-4.81 (m, 1 H), 4.595 (d, J = 4.0 Hz, 4 H), 2.43-2.25 (m, 8 H), 2.2 (s, 6H), 2.1-2.03 (m, 4 H), 1.81-1.73 (m, 2 H), 1.61-1.56 (m, 4 H), 1.48-1.47 (m, 4 H), 1.36-1.23 (m, 32 H), 0.86 (t, J = 8.0 Hz, 6 H).

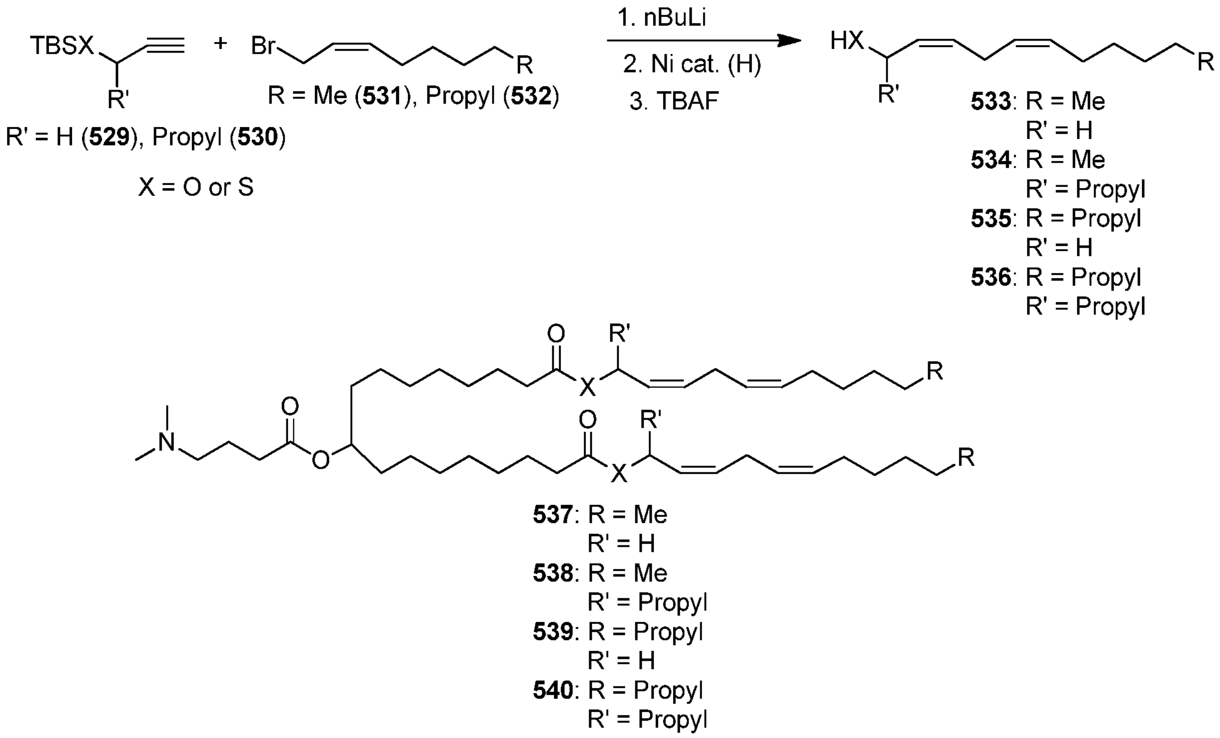
Compound **526:** (0.358 g, 60.9 %, R_f: 0.5, 5 % MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87-4.81 (m, 1 H), 4.07-3.95 (m, 4 H), 2.32-2.24 (m, 6 H), 2.2 (s, 6H), 1.80-1.69 (m, 4 H), 1.6-1.14 (m, 46 H), 0.88-0.84 (m, 24 H).

Compound **527:** (0.258 g, 56.8 %, R_f: 0.5, 5 % MeOH in DCM). Molecular weight for C47H91NO6 $(M+H)^+$ Calc. 766.23; Found: 766.7. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86-4.80 (m, 1 H), 4.12-4.02 (m, 4 H), 2.31-2.23 (m, 8 H), 2.19 (s, 6H), 1.80-1.72 (m, 2 H), 1.66-1.06 (m, 52 H), 0.87 (d, J = 8.0 Hz, 6 H), 0.84 (d, J = 8.0 Hz, 12 H).

Compound **528:** (0.3 g, 68.1 %, R_f: 0.5, 5 % MeOH in DCM). Molecular weight for C47H91NO6 $(M+H)^+$ Calc. 766.23; Found: 766.7. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86-4.80 (m, 1 H), 4.12-4.02 (m, 4 H), 2.31-2.21 (m, 8 H), 2.19 (s, 6H), 1.79-1.72 (m, 2 H), 1.66-0.98 (m, 52 H), 0.87 (d, J = 8.0 Hz, 6 H), 0.835 (d, J = 4.0 Hz, 12 H).

Example 28

Scheme 28:

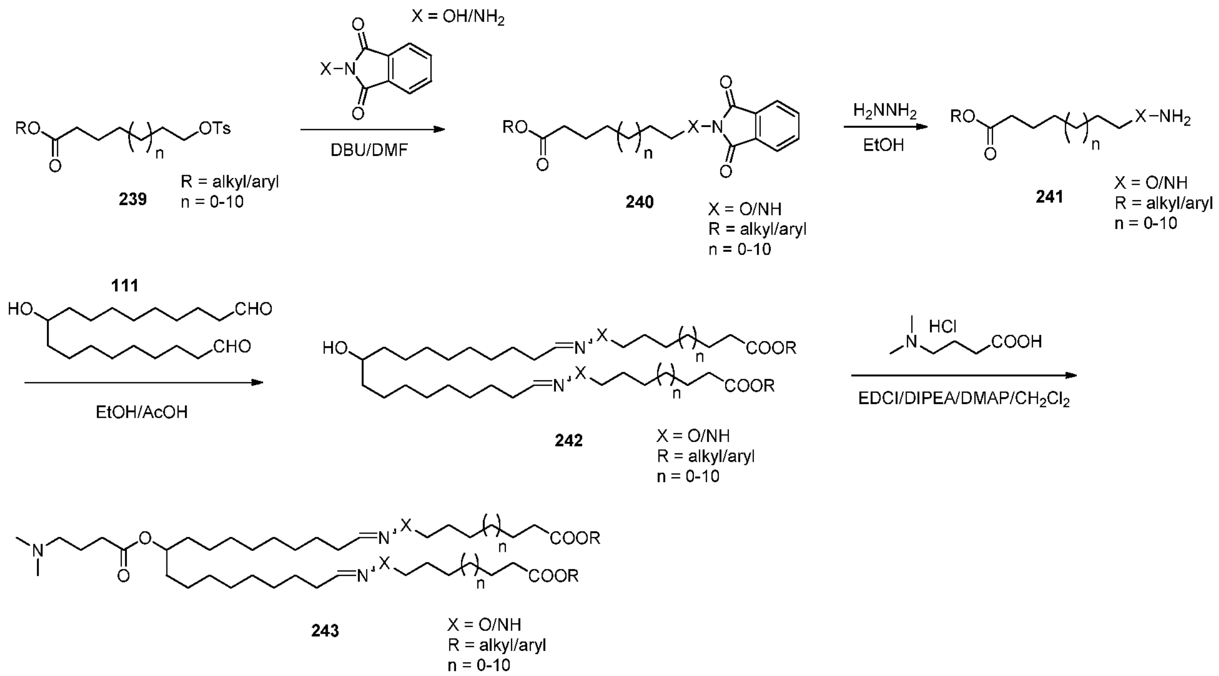


Synthesis of compounds **533**, **534**, **535** and **536**: The title compounds (1 mmol) are synthesized following the experimental procedure of compound **513** except de-silylation step and it is done using TBAF in THF at room temperature.

Synthesis of compounds **537**, **538**, **539** and **540**: The title compounds (1 mmol) are synthesized following the experimental procedure of compound **525**.

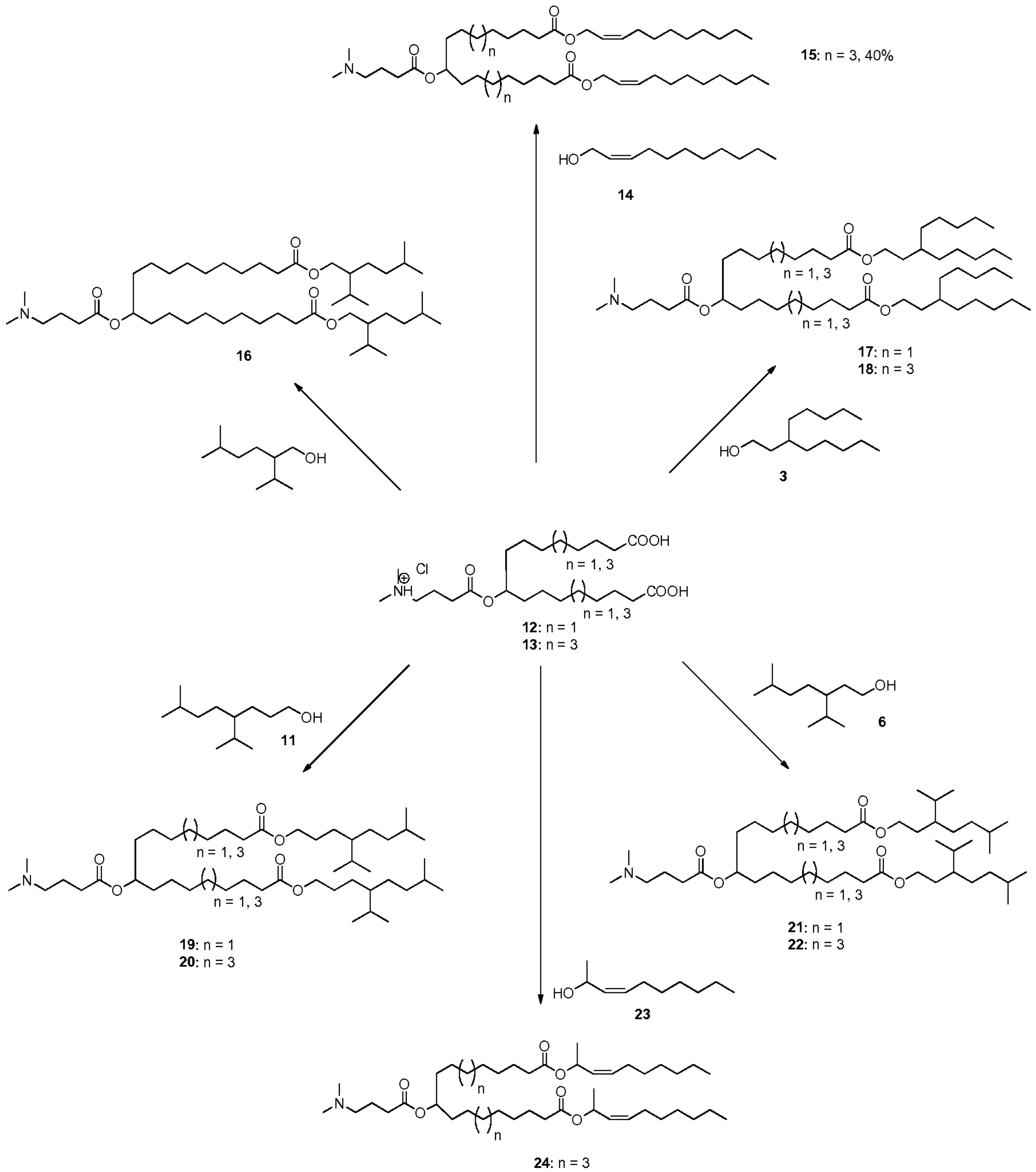
Example 29

Scheme 29



Compound **243** (X = O/NH, R = alkyl/aryl) can be synthesized as shown in Scheme 16-2. Tosyl group of **239** can be replaced with phthalimide group by nucleophilic substitution. After deprotection followed by coupling with **111** under acidic conditions, **242** can be synthesized. Standard esterification gives cationic lipid **243** and its analogs.

Example 30: Synthesis of ester-containing lipids



Compound 15: Compound **13** (503 mg, 1.0 mmol) was treated with **14** (469 mg, 3.0 mmol) in the presence of EDCI (2.30 g, 12.0 mmol), DMAP (235 mg, 1.92 mmol) and DIEA (8.34 mL, 47.9 mmol) in CH_2Cl_2 (50 mL) for 14 h. Aqueous work-up then column chromatography gave compound **15** (1.22 g, 1.54 mmol, 40%).

Molecular weight for $C_{49}H_{92}NO_6$ $(M+H)^+$ Calc. 790.6925, Found 790.7.

Compound 16: This compound was synthesized from **13** and tetrahydrolavandulol using a procedure analogous to that described for compound **15**. Yield: 0.358 g, 61 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87-4.81 (m, 1 H), 4.07-3.95 (m, 4 H), 2.32-2.24 (m, 6 H), 2.2 (s, 6H), 1.80-1.69 (m, 4 H), 1.6-1.14 (m, 46 H), 0.88-0.84 (m, 24 H).

Compound 17: This compound was synthesized from **12** (1.0 g, 2.15 mmol) and **3** (1.03 g, 5.16 mmol) using a procedure analogous to that described for compound **15**.
Yield: 856 mg (50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 – 4.79 (m, 1 H), 4.08 (t, J = 7.1 Hz, 4 H), 2.35 – 2.25 (m, 14 H), 1.89 – 1.76 (m, 2 H), 1.67 – 1.13 (m, 62 H), 0.88 (t, J = 7.0 Hz, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.08, 74.45, 63.08, 45.27, 34.76, 34.56, 34.28, 33.70, 32.61, 32.39, 29.54, 29.36, 29.28, 26.36, 25.47, 25.13, 22.83, 14.26. Molecular weight for $C_{49}H_{96}NO_6$ $(M+H)^+$ Calc. 794.7238, Found 794.6.

Compound 18: This compound was synthesized from **13** (1.0 g, 2.15 mmol) and **3** (1 g) using a procedure analogous to that described for compound **15**.
Yield: 1 g (59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 – 4.74 (m, 1H), 4.17 – 3.85 (m, 4H), 2.46 – 2.19 (m, 12H), 1.93 – 1.79 (m, 2H), 1.74 – 1.45 (m, 10H), 1.37 (d, *J* = 20.2 Hz, 2H), 1.35 – 1.13 (m, 44H), 0.88 (t, *J* = 6.9 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.19, 77.53, 77.21, 76.90, 63.12, 34.81, 34.66, 34.35, 33.76, 32.66, 32.45, 29.76, 29.73, 29.63, 29.48, 29.39, 26.42, 25.57, 25.23, 22.89, 14.32. Molecular weight for $C_{53}H_{103}NO_6$ $(M+H)^+$ Calc. 850.38, Found 850.7.

Compound 19: This compound was synthesized from **12** and **11** using a procedure analogous to that described for compound **15**.

Yield: 860 mg (51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 – 4.81 (m, 1 H), 4.04 (t, J = 6.8 Hz, 4 H), 2.37 – 2.17 (m, 14 H), 1.84 – 1.06 (m, 48 H), 0.93 – 0.78 (m, 24 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.06, 74.35, 65.51, 64.91, 59.05, 45.51, 43.77, 37.10, 34.55, 34.29, 32.55, 29.54, 29.37, 29.34, 29.28, 28.58, 28.19, 26.99, 26.74, 25.47, 25.15, 22.90, 22.82, 19.60, 19.41, 19.28. Molecular weight for $C_{47}H_{92}NO_6$ $(M+H)^+$ Calc. 766.6925, Found 766.5.

Compound 20: This compound was synthesized from **13** and **11** using a procedure analogous to that described for compound **15**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86 (p, J = 6.2 Hz, 1 H), 4.04 (t, J = 6.7 Hz, 4 H), 2.38 – 2.17 (m, 14 H), 1.84 – 1.07 (m, 56 H), 0.93 – 0.76 (m, 24 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.11, 173.46, 74.44, 64.90, 59.06, 45.51, 43.77, 37.11, 34.59, 34.32, 32.57, 29.71, 29.67, 29.57, 29.43, 29.34, 28.58, 28.20, 27.00, 26.75, 25.51, 25.20, 22.90, 22.82, 19.41, 19.28. Molecular weight for $C_{51}H_{100}NO_6$ $(M+H)^+$ Calc. 822.7551, Found 822.6.

Compound 21: This compound was synthesized from **12** and **6** using a procedure analogous to that described for compound **15**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 – 4.78 (m, 1 H), 4.15 – 3.98 (m, 4 H), 2.39 – 2.18 (m, 14 H), 1.84 – 1.11 (m, 44 H), 0.92 – 0.77 (m, 24 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.06, 173.44, 74.36, 63.73, 59.03, 45.48, 41.00, 36.98, 34.56, 34.29, 32.54, 29.60, 29.54, 29.49, 29.36, 29.28, 28.52, 25.47, 25.13, 23.15, 22.85, 22.81, 19.49, 18.89. Molecular weight for $C_{45}H_{88}NO_6$ $(M+H)^+$ Calc. 738.6612, Found 738.6.

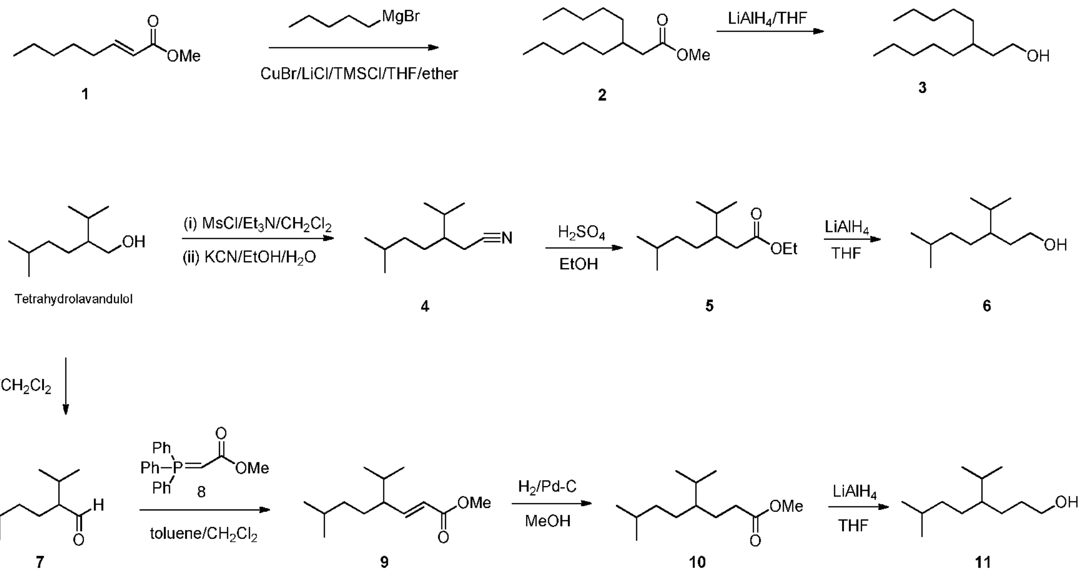
Compound 22: This compound was synthesized from **13** and **6** using a procedure analogous to that described for compound **15**.

Yield: 900 mg (57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 – 4.78 (m, 1H), 4.15 – 3.91 (m, 4H), 3.33 – 3.08 (m, 1H), 2.36 – 2.15 (m, 14H), 1.79 (dq, J = 14.3, 7.2 Hz, 2H), 1.74 – 1.55 (m, 8H), 1.55 – 1.37 (m, 9H), 1.35 – 0.95 (m, 36H), 0.96 – 0.61 (m, 27H). ^{13}C NMR (101 MHz$CDCl_3$) δ 174.16, 173.52, 77.54, 77.22, 76.91, 74.48, 63.76, 59.10, 45.55, 42.02, 41.04, 38.75, 37.09, 37.02, 34.65, 34.36, 32.62, 30.71, 29.75, 29.72, 29.64, 29.62, 29.53, 29.48, 29.44, 29.38, 28.56, 28.45, 25.56, 25.23, 23.59, 23.23, 22.90, 22.86, 19.54, 19.03, 18.94. Molecular weight for $C_{49}H_{95}NO_6$ $(M+H)^+$ Calc. 794.2817, Found 794.7.

Compound 24: This compound was synthesized from **13** and **23** using a procedure analogous to that described for compound **15**.

Yield: 0.567 g (30 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (p, J = 6.1 Hz, 1H), 4.20 – 3.93 (m, 4H), 2.41 – 2.18 (m, 13H), 1.92 – 1.72 (m, 2H), 1.56 (ddd, J = 27.4, 16.4, 5.8 Hz, 12H), 1.39 (s, 2H), 1.25 (s, 54H), 0.91 (dt, J = 13.7, 6.4 Hz, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.18, 173.51, 77.54, 77.23, 76.91, 74.50, 63.12, 59.10, 45.55, 34.81, 34.66, 34.38, 33.76, 32.67, 32.62, 32.45, 29.77, 29.73, 29.64, 29.49, 29.39, 26.42, 25.57, 25.24, 23.23, 22.89, 14.32. Molecular weight for $C_{47}H_{88}NO_6$ $(M+H)^+$ Calc. 762.6612, Found 762.5.

Example 31: Synthesis of alcohol components



Compound 2: Compound **2** was synthesized from **1** using a procedure analogous to that described in *Journal of the Organic Chemistry*, 2009, 1473.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3 H), 2.23 (d, J = 6.9 Hz, 2 H), 1.84 (brs, 1 H), 1.27 (d, J = 11.5 Hz, 16 H), 0.88 (t, J = 6.8 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.29, 51.49, 39.25, 35.22, 34.00, 32.24, 26.34, 22.77, 14.22.

Compound 3: To a suspension of $LiAlH_4$ (2.84 g, 74.9 mmol) in THF (85 mL) was added a solution of compound **2** (8.55 g, 37.4 mmol) in THF (25 mL). The reaction mixture was refluxed overnight. Aqueous workup then column chromatography gave pure compound **3** (7.35 g, 36.7 mmol, 98%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, J = 7.0 Hz, 2 H), 1.59 – 1.12 (m, 19 H), 0.88 (t, J = 6.9 Hz, 6 H).

Compound 4: Tetrahydrolavandulol (10.1 g, 63.8 mmol) was treated with methansulfonyl chloride (6.38 mL) in CH_2Cl_2 (200 mL) and Et_3N (17.6 mL). Aqueous workup gave the crude mesylate, which was treated with KCN (4.98 g, 76.5 mmol) in EtOH (90 mL) and H_2O (10 mL). Aqueous workup then column chromatography gave pure compound **4** (8.36 g, 50.0 mmol, 72%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 – 2.23 (m, 2 H), 1.86 – 1.78 (m, 1 H), 1.59 – 1.42 (m, 3 H), 1.40 – 1.07 (m, 3 H), 0.93 – 0.89 (m, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 119.73, 41.69, 36.46, 30.10, 28.44, 28.33, 22.82, 22.59, 19.62, 19.11, 19.05.

Compound 6: The cyano derivative **4** was converted to the ethyl ester under acidic conditions to give compound **5** and the ester was reduced by $LiAlH_4$ in THF to give compound **6**.

Compound 7: Tetrahydrolavandulol (98.1 g, 51.2 mmol) was oxidized with PCC (16.6 g, 76.8 mmol) in CH_2Cl_2 (200 mL). Aqueous workup then column chromatography gave pure compound **7** (6.19 g, 39.6 mmol, 77%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (d, J = 3.1 Hz, 1 H), 2.05 – 1.79 (m, 1 H), 1.71 – 1.36 (m, 4 H), 1.23 – 1.04 (m, 2 H), 1.02 – 0.82 (m, 12 H).

Compound 9: To a solution of compound **7** (2.0 g, 12.8 mmol) in toluene (40 mL) and CH_2Cl_2 (18 mL) and was added **8** (3.96 g, 11.8 mmol). The mixture was heated at 70 ^{o}C overnight. Column chromatography gave pure compound **9** (1.40 g, 6.59 mmol, 51%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (dd, J = 15.6, 9.9 Hz, 1 H), 5.76 (d, J = 15.6 Hz, 1 H), 3.73 (s, 3 H), 1.97 – 1.83 (m, 1 H), 1.72 – 1.64 (m, 1 H), 1.54 – 1.40 (m, 2 H), 1.37 – 1.22 (m, 1 H), 1.18 – 0.97 (m, 2 H), 0.94 – 0.78 (m, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.19, 152.54, 121.70, 51.53, 49.66, 36.95, 31.76, 29.49, 28.29, 22.92, 22.54, 20.84, 19.24.

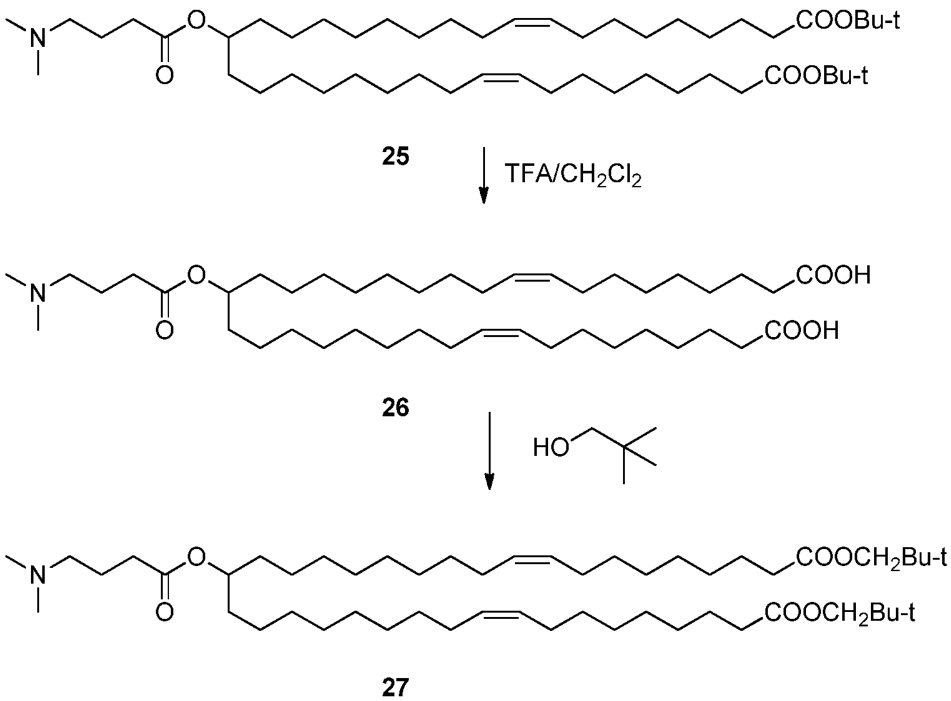
Compound 10: To a solution of compound **9** (1.0 g, 4.71 mmol) in MeOH (15 mL) was added Pd-C (125 mg). The mixture was stirred under H_2 atmosphere overnight. The mixture was filtered over Celite then evaporated to give pure compound **10** (924 mg, 4.31 mmol, 92%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3 H), 2.41 – 2.16 (m, 2 H), 1.74 – 1.57 (m, 2 H), 1.57 – 1.42 (m, 2 H), 1.33 – 1.02 (m, 5 H), 0.88 – 0.83 (m, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.78, 51.62, 43.71, 36.97, 32.69, 29.23, 28.56, 27.94, 25.92, 22.85, 22.79, 19.32, 19.19.

Compound 11: To a suspension of $LiAlH_4$ (444 mg, 11.7 mmol) in THF (12 mL) was added a solution of compound **10** (1.25 g, 5.83 mmol) in THF (8 mL). The reaction mixture was refluxed overnight. Aqueous workup gave the crude compound **11** (1.1 g) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (t, J = 6.7 Hz, 2 H), 1.74 – 1.66 (m, 1 H), 1.60 – 1.45 (m, 3 H), 1.37 – 1.05 (m, 7 H), 0.88 – 0.82 (m, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.75, 44.00, 37.16, 31.22, 29.40, 28.61, 28.28, 26.62, 22.90, 22.82, 19.43, 19.28.

Example 32: Synthesis of ester-containing lipids



Compound 26: Compound **25** (840 mg, 1.03 mmol) was stirred in TFA (9 mL) and CH_2Cl_2 (36 mL) for 3 h at room temperature. Evaporation of the solvents and co-evaporation with toluene 3 times gave compound **26**.

Molecular weight for $C_{43}H_{80}NO_6$ $(M+H)^+$ Calc. 706.5986, Found 706.4.

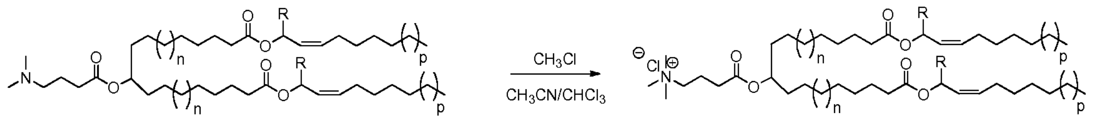
Compound 27: Compound **26** from the previous step was treated with 2,2-dimethylpropanol (363 mg, 4.12 mmol) in the presence of EDCI (592 mg, 3.09 mmol), DMAP (50 mg, 0.412 mmol) and DIEA (1.44 mL, 8.24 mmol) in CH_2Cl_2 (10 mL) for 14 h. Aqueous work-up then column chromatography gave compound **27** (575 mg, 0.679 mmol, 66%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 – 5.28 (m, 4 H), 4.91 – 4.81 (m, 1 H), 3.76 (s, 4 H), 2.34 – 2.27 (m, 8 H), 2.22 (s, 6 H), 2.03 – 1.97 (m, 8 H), 1.83 – 1.26 (m, 50 H), 0.94 (s, 18 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.14, 173.53, 130.09, 129.92, 74.41, 73.72, 59.12, 45.61, 34.60, 34.32, 32.64, 31.45, 29.93, 29.85, 29.71, 29.68, 29.48, 29.32, 29.28, 27.39, 27.33, 26.62, 25.52, 25.22, 23.32.

Molecular weight for $C_{53}H_{100}NO_6$ $(M+H)^+$ Calc. 846.7551, Found 846.5.

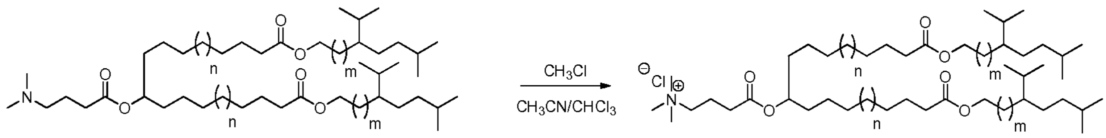
Example 33: Synthesis of quaternary lipids

A. The amino lipids synthesized in Examples 31 and 32 can be converted to the corresponding quaternary lipids as shown below by treatment with CH_3Cl in CH_3CN and $CHCl_3$.



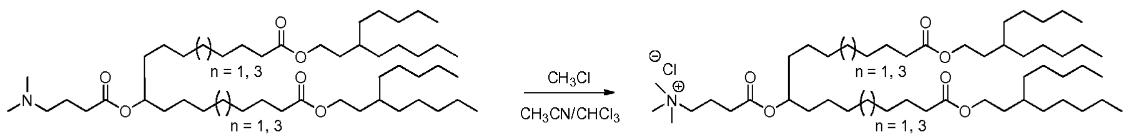
15: n = 3, p =3, R = H
24: n = 3, p = 1, R = Me

15Q: n = 3, p =3, R = H
24Q: n = 3, p = 1, R = Me



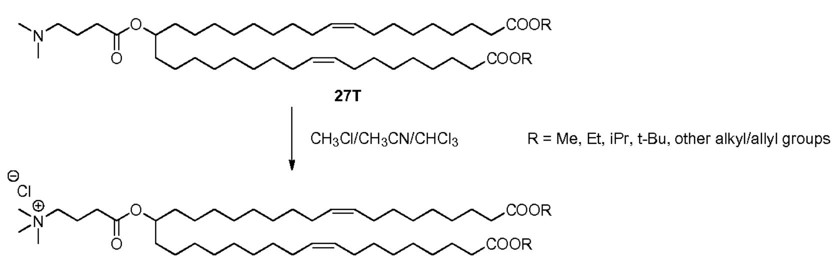
16: m = 0, n = 3
19: m = 2, n = 1
20: m = 2, n = 3
21: m = 1, n = 1
22: m = 1, n = 3

16Q: m = 0, n = 3
19Q: m = 2, n = 1
20Q: m = 2, n = 3
21Q: m = 1, n = 1
22Q: m = 1, n = 3



17: n = 1
18: n = 3

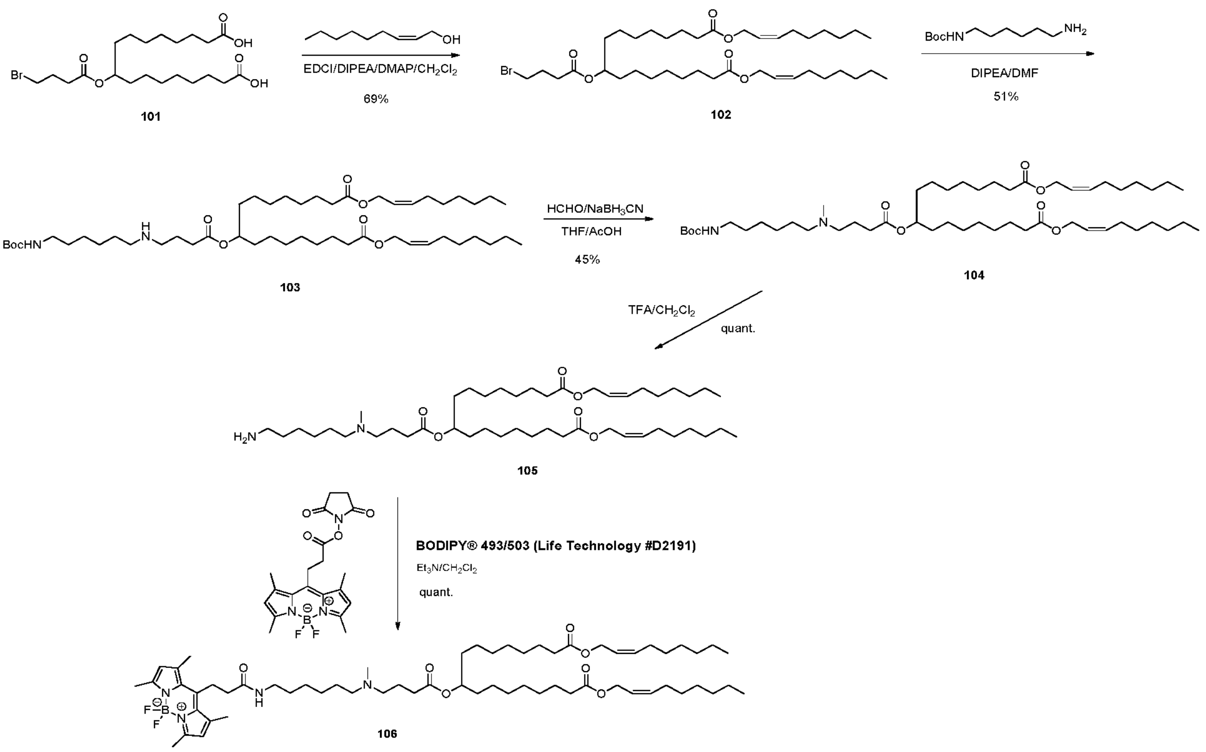
17Q: n = 1
18Q: n = 3



27TQ

B. Synthesis of BODIPY-lipid conjugates

Synthesis of BODIPY-labeled lipid



Compound 102: To a solution of compound **101** (2.00 g, 4.30 mmol) and *cis*-2-nonen-1-ol (1.81 mL, 10.7 mmol) in CH_2Cl_2 (20 mL) were added diisopropylethylamine (3.00 mL, 17.2 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (2.06 g, 10.7 mmol) and DMAP (106 mg, 0.868 mmol). The reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed with saturated $NaHCO_3$ aq. (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude was purified by silica gel column chromatography (0-5% EtOAc in Hexane) to give compound **102** (2.11 g, 2.96 mmol, 69%, R_f = 0.45 developed with 10% EtOAc in Hexane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.67 – 5.61 (m, 2 H), 5.54 – 5.49 (m, 2 H), 4.89 – 4.84 (m, 1 H), 4.62 (d, J = 6.5 Hz, 4 H), 3.46 (t, J = 6.5 Hz, 2 H), 2.48 (t, J = 7.3 Hz, 2 H), 2.30 (t, J = 7.5 Hz, 4 H), 2.20 – 2.14 (m, 2 H), 2.12 – 2.04 (m, 4 H), 1.63 – 1.60 (m, 4 H), 1.51 – 1.50 (m, 4 H), 1.37 – 1.27 (m, 32 H), 0.88 (t, J = 6.8 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.90, 172.45, 135.58, 123.51, 74.74, 60.36, 34.47, 34.24, 32.93, 32.91, 31.83, 29.54, 29.48, 29.31, 29.21, 29.01, 28.03, 27.70, 25.43, 25.08, 22.76, 14.23.

Molecular weight for $C_{39}H_{69}BrNaO_6$ $(M+Na)^+$ Calc. 735.42, Found 735.2.

Compound 103: To a solution of **102** (2.11 g, 2.96 mmol) in DMF (20 mL) was added a solution of *N*-Boc-1,6-diaminohexane (670 mg, 3.10 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred for 18 hours at room temperature. Then additional *N*-Boc-1,6-diaminohexane (160 mg, 0.740 mmol) in DMF (1 mL) was added and the mixture was stirred for 12 hour. The reaction was quenched by adding saturated $NaHCO_3$ aq. (100 mL) then extracted with Et_2O (150 mL x 3). The organic layer was separated and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude was purified by silica gel column chromatography (5% MeOH in CH_2Cl_2, $R_f = 0.24$) to give **103** (1.28 g, 1.51 mmol, 51%).

^1H NMR (400 MHz, $CDCl_3$) δ 5.67 – 5.61 (m, 2 H), 5.55 – 5.50 (m, 2 H), 4.88 – 4.81 (m, 1 H), 4.61 (d, J = 6.8 Hz, 4 H), 4.54 (brs, 1 H), 3.11 – 3.08 (m, 2 H), 2.67 – 2.59 (m, 4 H), 2.35 (t, J = 7.4 Hz, 2 H), 2.29 (t, J = 7.6 Hz, 4 H), 2.10 – 2.07 (m, 4 H), 1.84 – 1.81 (m, 4 H), 1.63 – 1.57 (m, 4 H) 1.50 – 1.47 (m, 8 H), 1.44 (s, 9 H), 1.38 – 1.27 (m, 34 H), 0.88 (t, J = 6.8 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.90, 173.53, 135.57, 123.50, 74.49, 60.36, 49.82, 49.29, 40.64, 34.47, 34.24, 32.68, 31.83, 30.16, 29.89, 29.54, 29.50, 29.33, 29.23, 29.01, 28.58, 27.69, 27.11, 26.80, 25.44, 25.37, 25.09, 22.76, 14.23.

Molecular weight for $C_{50}H_{93}N_2O_8$ $(M+H)^+$ Calc. 849.69, Found 849.5.

Compound 104: To a solution of **103** (1.16 g, 1.37 mmol) in THF (20 mL) were added formaldehye (37 wt. % in H_2O, 0.306 mL, 4.11 mmol), sodium cyanoborohydride (1 M solution in THF, 2.06 mL, 2.06 mmol) and acetic acid (0.008 mL, 0.137 mmol) at 0 °C. The mixture was stirred at room temperature for 17 hours. The reaction was quenched by adding saturated $NaHCO_3$ aq. (50 mL) then extracted with Et_2O (100 mL x 3). The organic layer was separated and dried over anhydrous $MgSO_4$. After filtration and concentration, the crude was purified by silica gel column chromatography (8% MeOH in CH_2Cl_2, $R_f = 0.46$) to give **104** (531 mg, 0.615 mmol, 45%).

^1H NMR (400 MHz, $CDCl_3$) δ 5.66 – 5.60 (m, 2 H), 5.53 – 5.47 (m, 2 H), 4.86 – 4.80 (m, 1 H), 4.61 – 4.59 (m, 5 H), 3.12 – 3.07 (m, 2 H), 2.89 – 2.78 (m, 4 H), 2.62 (s, 3 H), 2.40 (t, J = 6.8 Hz, 2 H), 2.28 (t, J = 7.4 Hz, 4 H), 2.11 – 2.06 (m, 4 H), 1.99 – 1.92 (m, 2 H), 1.69 – 1.27 (m, 57 H), 0.87 (t, J = 6.8 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.86, 172.45, 156.18, 135.55, 123.45, 75.24, 60.32, 56.68, 55.83, 40.72, 40.36, 34.40, 34.09, 31.79, 31.29, 29.92, 29.49, 29.41, 29.26, 29.17, 28.96, 28.55, 27.65, 26.49, 26.30, 25.41, 25.02, 24.79, 22.71, 20.12, 14.19.

Molecular weight for $C_{51}H_{95}N_2O_8$ $(M+H)^+$ Calc. 863.71, Found 863.6.

Compound 105: To a solution of compound **104** (525 mg, 0.608 mmol) in CH_2Cl_2 (8 mL) was added trifluoroacetic acid (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and at room temperature for 3 hours. The reaction mixture was evaporated and co-evaporated with toluene 3 times then dried *in vacuo* overnight to give compound **105** (603 mg, 0.603 mmol calculated as 2 TFA salt, quantitatively, R_f = 0.24 developed with 8% MeOH in CH_2Cl_2).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (brs, 1 H), 5.68 – 5.61 (m, 2 H), 5.55 – 5.49 (m, 2 H), 4.87 – 4.81 (m, 1 H), 4.62 (d, J = 6.8 Hz, 4 H), 4.28 (brs, 3 H), 3.20 – 3.02 (m, 6 H), 2.82 (d, J = 4.0 Hz, 3 H), 2.45 – 2.40 (m, 2 H), 2.30 (t, J = 7.4 Hz, 4 H), 2.12 – 2.00 (m, 6 H), 1.78 – 1.22 (m, 52 H), 0.88 (t, J = 6.8 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.04, 172.08, 161.84, 161.47, 135.63, 123.44, 117.60, 114.71, 75.56, 60.41, 55.69, 55.27, 39.94, 39.64, 34.44, 34.06, 31.82, 30.72, 29.53, 29.43, 29.28, 29.19, 29.00, 27.69, 26.58, 25.42, 25.27, 25.05, 24.60, 23.06, 22.75, 19.00, 14.22.

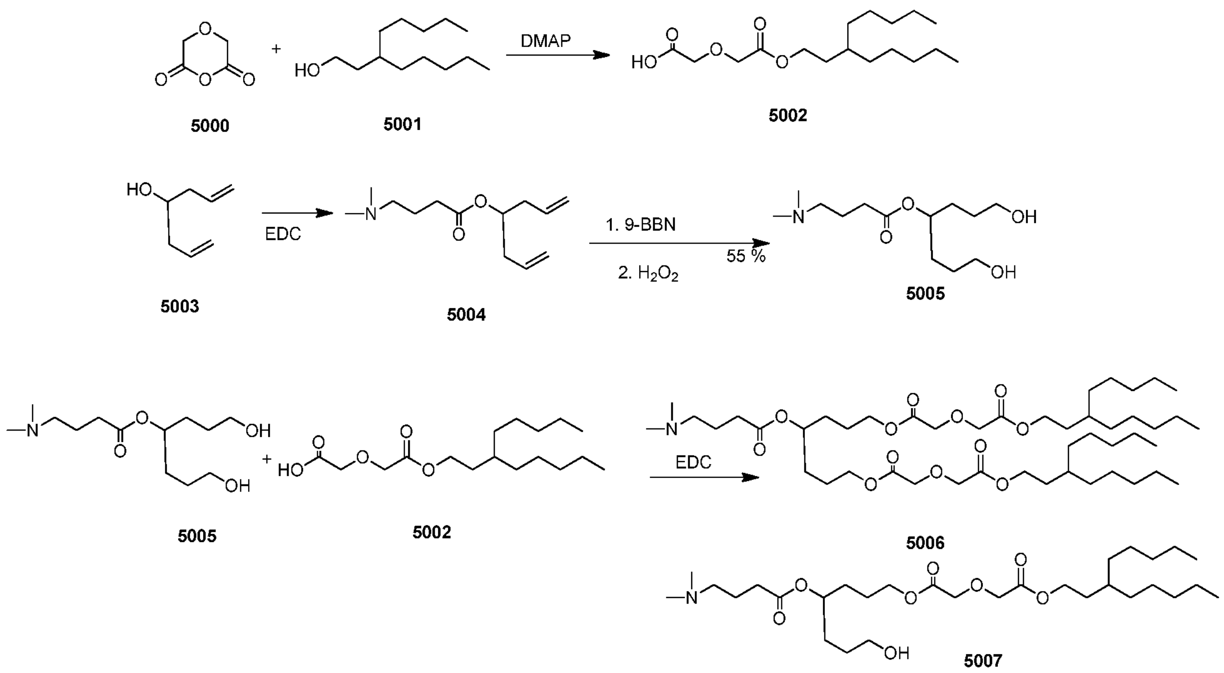
Molecular weight for $C_{46}H_{87}N_2O_6$ $(M+H)^+$ Calc. 763.66, Found 763.4.

Compound 106: To a solution of **105** (23.8 mg, 0.0240 mmol, calculated as 2TFA salt) in CH_2Cl_2 (1 mL) and Et_3N (0.050 mL, 0.360 mmol) was added a solution of BODIPY® 493/503 (10 mg, 0.0240 mmol, Life Technology #D2191) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 1 h. The reaction mixture was loaded onto silica gel column chromatography and eluted with 0-5% MeOH in CH_2Cl_2. The product color fractions were collected (5% MeOH in CH_2Cl_2, R_f = 0.36) to give **106** (26 mg, 0.024 mmol, quantitatively).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 2 H), 5.67 – 5.61 (m, 2 H), 5.54 – 5.48 (m, 2 H), 4.85 – 4.82 (m, 1 H), 4.61 (d, J = 6.8 Hz, 4 H), 3.37 – 3.32 (m, 2 H), 3.27 – 3.22 (m, 2 H), 2.51 – 2.44 (m, 17 H),, 2.34 – 2.27 (m, 8 H), 2.12 – 2.06 (m, 4 H), 1.60 – 1.21 (m, 52 H), 0.88 (t, J = 6.8 Hz, 6 H).

Molecular weight for $C_{62}H_{104}BF_2N_4O_7$ $(M+H)^+$ Calc. 1065.80, Found 1065.5.

Example 34: Multi-ester containing Lipids and Acetal linked lipids

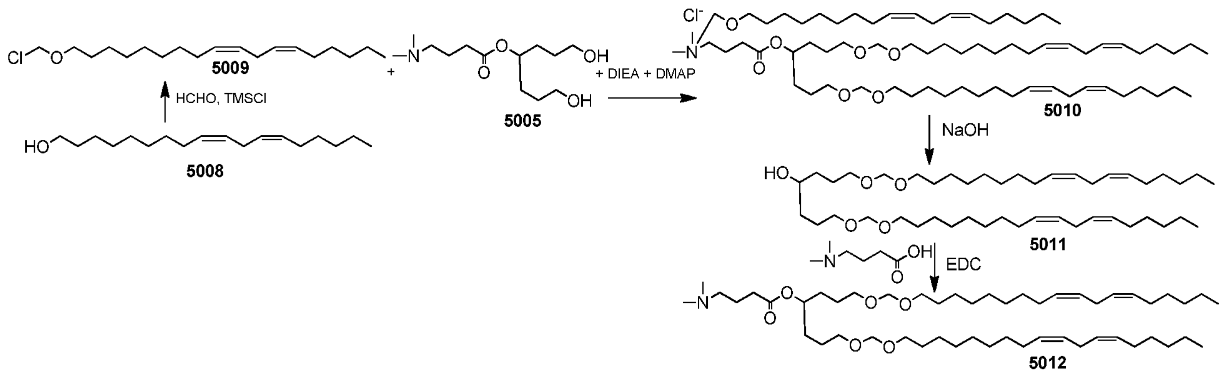


Synthesis of compound **5002**: To a stirred solution of alcohol **5001** (1.0 g, 5.15 mmol), Glycolic anhydride 5000 (5.66 mmol) in DCM (20 mL) was added DMAP (1.26 g, 10.41 mmol) and stirred at room temperature for 48 h. The reaction mixture was concentrated followed by column purification gave the corresponding product **5002** (1.4 g, 86 %) as DMAP salt. LCMS: Calculated: 316.22 (M^+), Found: 315.1 (M^+-1).

Synthesis of compound **5004**: To a stirred solution of alcohol 5003 (5.0 g, 44.6 mmol), 4-(Dimethylamino)butyric acid hydrochloride (8.1 g, 48.3 mmol) and EDC (10.3 g, 53.6 mmol) in DCM (100 mL) was added DIEPA (23 g, 178.3 mmol) and stirred at room temperature overnight. After usual work up, the crude product was purified by column chromatography (9.0 g, 90 %).

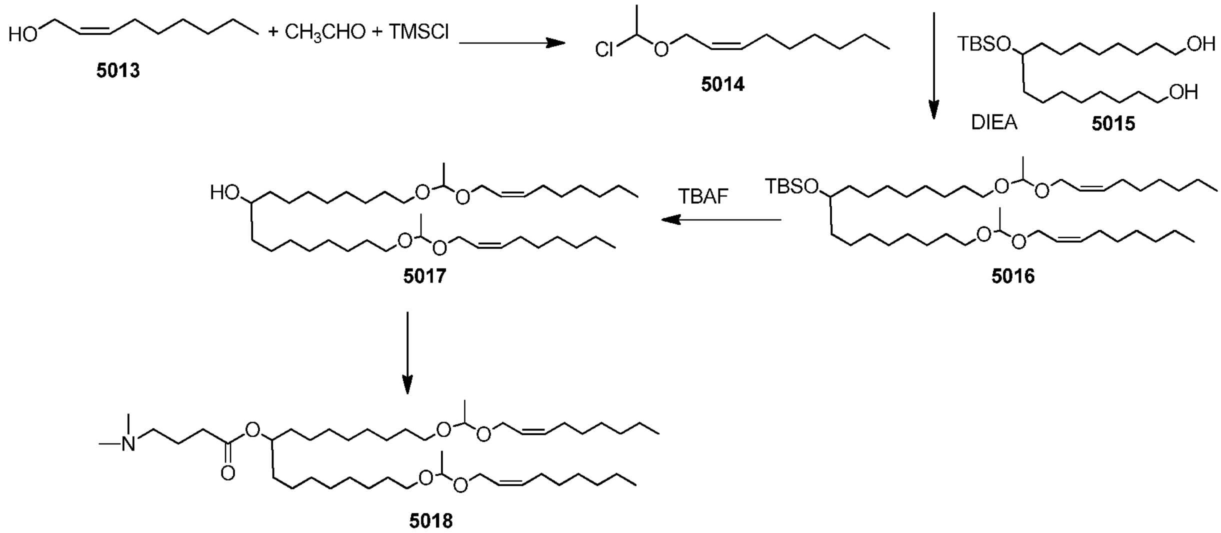
Synthesis of compound **5005**: To a stirred solution of diene 5004 (4.0 g, 18 mmol) in 10 mL of THF was added 9-BBN and stirred overnight. To the above solution was added 6.6 mL of 3M NaOAc and 7.4 mL of 30 % H_2O_2 at 0-5° C. The reaction mixture was stirred at room temperature overnight. After usual work up, the crude material was purified by column chromatography to get 5005 (2.6 g, 55 %) as viscous oil. LCMS: Calculated: 261.19 (M^+), Found: 262.1 (M^++1).

Synthesis of compound **5006** and **5007**: To a stirred solution of diene 5005 (260 mg, 1 mmol), acid 5002 (1.0 g, 2.28 mmol), EDC (387 mg, 2 mmol) in 10 mL of DCM was added DIEA (516 mg, 4 mmol) and stirred overnight. After usual work up, the crude material was purified by column chromatography to get 5006 (0.1 g, 12 %) and 5007 (0.2 g, 36 %). LCMS for compound **5006**: Calculated: 857.62 (M^+), Found: 858.5 (M^++1), 880.5 (M^++Na). LCMS for compound **5007**: Calculated: 559.4 (M^+), Found: 560.4 (M^++1).



Synthesis of compound **5011**: To a stirred solution of alcohol **5008** (2.66 g 10 mmol) in 5 mL of Chlorotrimethylsilane was added paraformaldehyde (0.3 g, 10 mmol) and stirred at room temperature overnight. The excess Chlorotrimethylsilane was evaporated followed by drying under reduced pressure gave the corresponding product **5009** and used for next step without purification. The compound **5009** was added dropwise to the solution of diol (261 mg, 1 mmol), DIEA (2.5 g, 19.4 mmol) and DMAP (20 mg, 0.16 mmol) in DCM (10 mL) and stirred overnight. Concentration of the solvent gave the crude product **5010**, which was dissolved in 5 mL of THF and 2 mL of 1N NaOH was added and stirred for 2 days at room temperature. After usual work up, the crude material was purified by column chromatography to get the corresponding product **5011** (200 mg, 28 %). LCMS for compound **5010**: Calculated: 1131.95 (M^+), Found: 1096.98 (M^+ -Cl^-)). LCMS for compound **5011**: Calculated: 704.63 (M^+), Found: 727.5 (M^++Na).

Synthesis of compound **5012**: To a stirred solution of alcohol 5011 (200 mg, 0.284 mmol), 4-(Dimethylamino)butyric acid hydrochloride (103 mg, 0.57 mmol), EDC (109 mg, 0.57 mmol) in 10 mL of DCM was added DIEA (294 mg, 4 mmol) and stirred overnight. After usual work up, the crude material was purified by column chromatography to get 5012 (190 mg, 85 %). LCMS for compound **5012**: Calculated: 817.72 (M^+), Found: 818.5 (M^++Na).



Synthesis of compound **5016**: To a stirred solution of alcohol **5013** (1.0 g 7.03 mmol) in 5 mL of Chlorotrimethylsilane was added acetaldehyde (0.3 g, 7.03 mmol) and stirred at room temperature for 2 h. The excess Chlorotrimethylsilane was evaporated followed by drying under reduced pressure gave the corresponding product **5014** and used for next step without purification. The compound **5014** was added dropwise to the solution of diol **5015**(223 mg, 0.55 mmol), DIEA (2 mL g, 11.5 mmol) and DMAP (20 mg, 0.16 mmol) in DCM (10 mL) and stirred overnight. 10 mL of water was added followed by extraction with DCM (3 X 30 mL), washed with water, saturated $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. Concentration of the solvent gave the crude product, which was used for the next step without purification. LCMS for compound **5016**: Calculated: 738.66 (M^+), Found: 761.5 (M^++Na).

Synthesis of compound **5017**: To a stirred solution of alcohol 5016 in 5 mL of THF was added 0.54 mL of 1M TBAF in THF (0.54 mmol) and stirred for 2 days at room temperature. After usual work up, the crude material was purified by column chromatography to get 5017. However, it contains some inseparable impurity and hence used for next step without further purification. LCMS for compound **5017**: Calculated: 624.57 (M^+), Found: 647.5 (M^++Na).

Synthesis of compound **5018**: To a stirred solution of alcohol 5017 (0.55 mmol), 4-(Dimethylamino)butyric acid hydrochloride (116 mg, 0.64 mmol), EDC (123 mg, 0.64 mmol) in 10 mL of DCM was added DIEA (165 mg, 1.28 mmol) and stirred for 2 days. After usual work up, the crude material is purified by column chromatography (0-10 % MeOH in 1 % Et_3N containing DCM) to get 5018 (300 mg, 75 % from 5015). LCMS for compound **5018**: Calculated: 737.65 (M^+), Found: 738.6 (M^++1), 760.5 (M^++Na^+).

Example 35: Preparation of Lipid Nanoparticles

The cationic lipids described herein are used to formulate liposomes containing the AD-1661 duplex (shown in the table below) using an in-line mixing method as described in International Publication No. WO 2010/088537, which is incorporated by reference in its entirety. The lipid nanoparticles had the formulation shown in the table below.

Component	Mole Percentage (Based on 100% of the lipid components in the LNP)
Cationic lipid	50%
Distearoylphosphatidylcholine (DSPC)	10%
Cholesterol	38.5%
1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG) (with an average PEG molecular weight of 2000)	1.5%
siRNA (AD-1661)	-

The siRNA AD-1661 duplex has the sequence shown below.

Duplex	Sequence 5'-3'	SEQ ID NO:	Target
AD-1661	GGAfUfCAfUfCfUfCAAGfUfCfUfUAfCdTsdT	1	FVII
	GfUAAGAfCfUfUGAGAfUGAfUfCfCdTsdT	2	

Lower case is 2'OMe modification and Nf is a 2'F modified nucleobase, dT is deoxythymidine, s is phosphothioate

The lipid nanoparticles was prepared as follows. Cationic lipid, DSPC, cholesterol, and PEG-DMG in the ratio recited in the table above were solubilized in ethanol at a total lipid concentration of 25 mg/mL.

A siRNA stock solution was prepared by solubilizing the siRNA AD-1661 in a low pH acetate or citrate buffer (pH=4) at 0.8 mg/mL.

The stock solutions should be completely clear and the lipids should be completely solubilized before combining with the siRNA. Therefore, if it was determined appropriate, the stock solutions were heated to completely solubilize the lipids.

The individual stock solutions were combined by pumping each solution to a T-junction (i.e., by in-line mixing). Specifically, the ethanol solution (at 5 ml/min, via 0.01 in. PEEK tube) and aqueous buffer solution (at 15 mL/min, via 0.02 in. PEEK tube) were mixed through a T-junction (PEEK Tee body, IDEX).

After the T-junction a single tubing is placed where the combined stream will emit. Ethanol is removed and exchanged for PBS by dialysis. The lipid formulations are then concentrated using centrifugation or diafiltration to an appropriate working concentration.

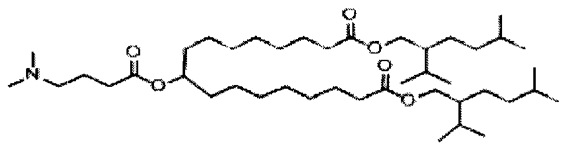
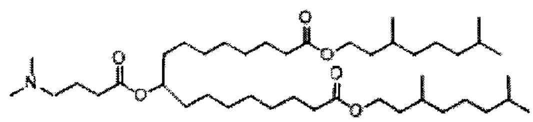
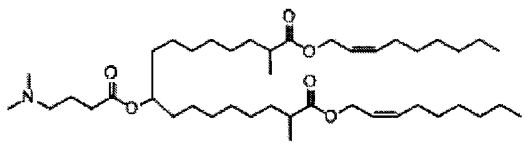
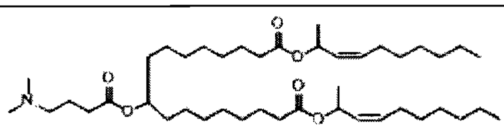
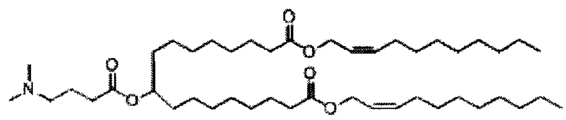
Lipid nanoparticles containing the cationic lipids listed in the table in Example 36 were prepared as described above.

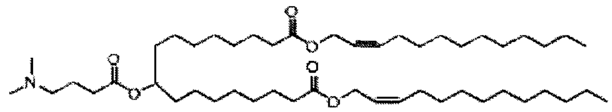
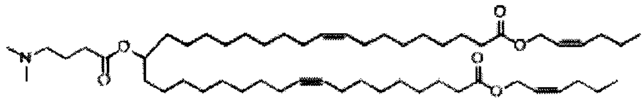
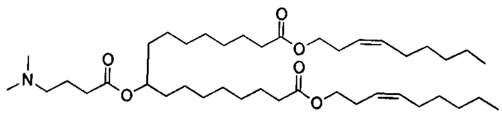
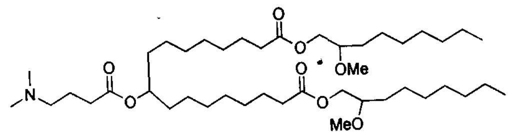
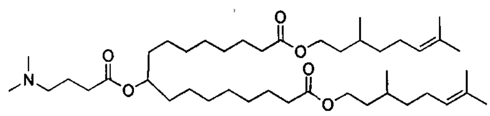
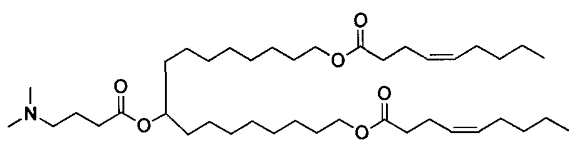
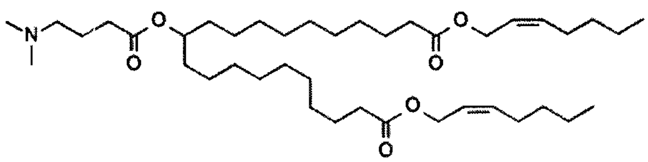
Example 36: Efficacy of Lipid Nanoparticles

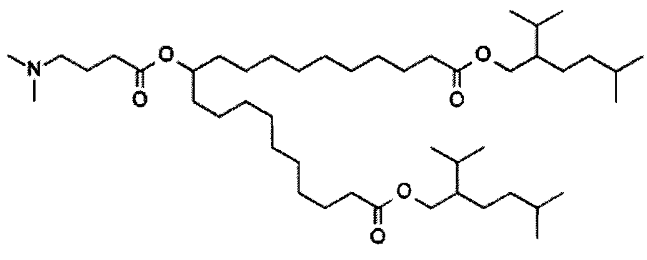
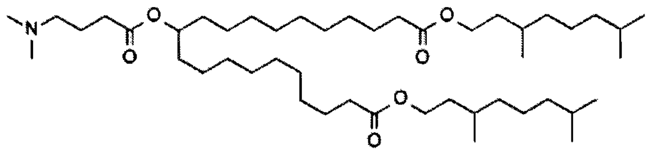
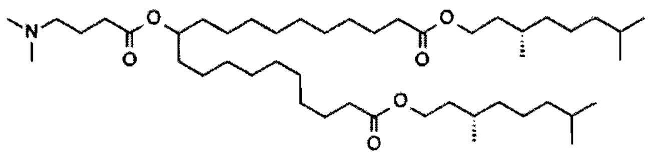
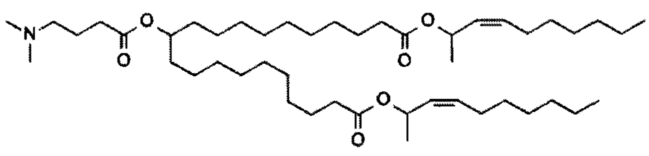
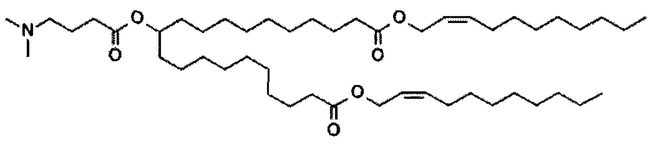
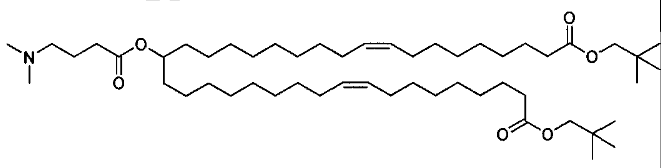
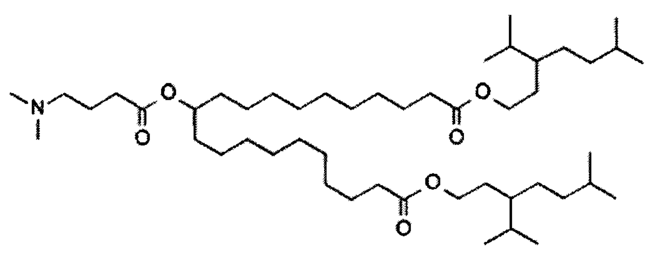
Factor VII (FVII), a prominent protein in the coagulation cascade, is synthesized in the liver (hepatocytes) and secreted into the plasma. FVII levels in plasma can be determined by a simple, plate-based colorimetric assay. As such, FVII represents a convenient model for determining siRNA-mediated downregulation of hepatocyte-derived proteins.

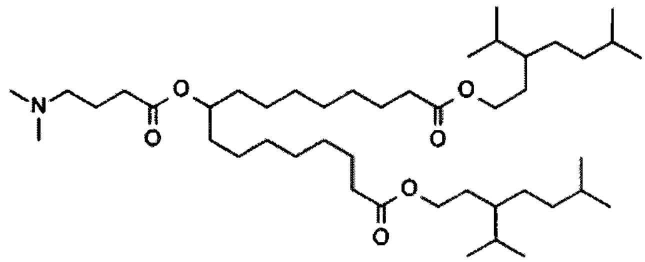
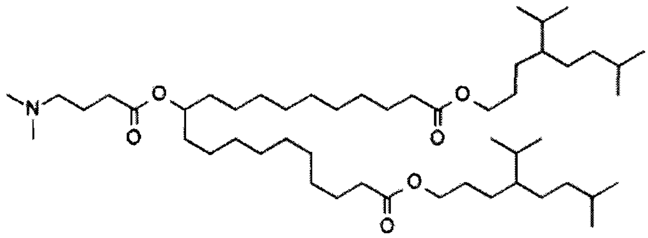
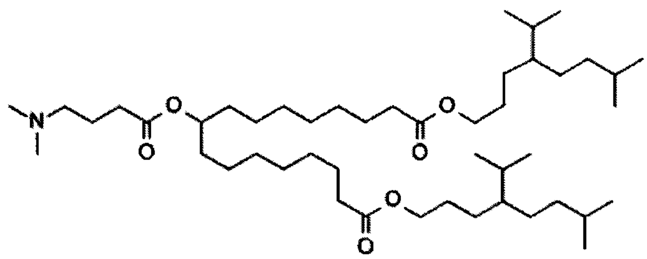
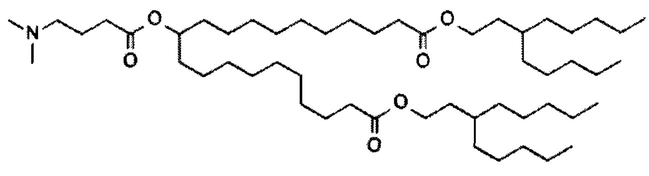
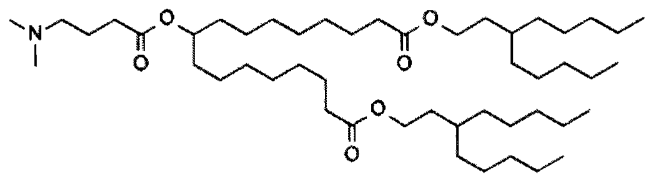
Test formulations of the lipid nanoparticles prepared in Example 35 were initially assessed for their FVII knockdown in female 7-9 week old, 15-25g, female C57Bl/6 mice at 0.1, 0.3, 1.0 and 5.0 mg/kg with 3 mice per treatment group. All studies included animals receiving either phosphate-buffered saline (PBS, control group) or a benchmark formulation. Formulations were diluted to the appropriate concentration in PBS immediately prior to testing. Mice were weighed and the appropriate dosing volumes calculated (10 μl/g body weight). Test and benchmark formulations as well as PBS (for control animals) were administered intravenously via the lateral tail vein. Animals were anesthetised 24 hours later with an intraperitoneal injection of ketamine/xylazine and 500-700 μl of blood was collected by cardiac puncture into serum separator tubes (BD Microtainer). Blood was centrifuged at 2,000 x g for 10 minutes at 15° C and serum was collected and stored at -70° C until analysis. Serum samples were thawed at 37° C for 30 minutes, diluted in PBS and aliquoted into 96-well assay plates. Factor VII levels were assessed using a chromogenic assay (Biophen FVII kit, Hyphen BioMed) according to the manufacturer's instructions and absorbance was measured in a microplate reader equipped with a 405 nm wavelength filter. Plasma FVII levels were quantified and ED_{50} values (dose resulting in a 50% reduction in plasma FVII levels compared to control animals) were calculated using a standard curve generated from a pooled sample of serum from control animals. Those formulations of interest showing high levels of FVII knockdown (ED_{50} << 0.1 mg/kg) were re-tested in independent studies at a lower dose range to confirm potency and establish ED_{50} levels.

The following table shows ED_{50} values for some of the cationic lipids described herein. Two asterisks (**) indicates an ED_{50} value between 0.001 and 0.10. One asterisk (*) indicates an ED_{50} value greater than 0.10.

ED_{50}	Cationic Lipid
**	
**	
**	
**	
**	

ED_{50}	Cationic Lipid
**	
**	
**	
*	
**	
*	
**	
**	
**	

ED_{50}	Cationic Lipid
**	
**	
**	
**	
**	
**	
**	

ED50	Cationic Lipid
**	
**	
**	
**	
**	

Example 37: Hydrophobicity and Stability

The logP values for the biodegrabable cationic lipids listed in the table below were calculated using the software available at http://www.molinspiration.com/services/logp.html from Molinspiration Cheminformatics of Slovensky Grob, Slovak Republic.

Furthermore, the HPLC retention time for each biodegradable cationic lipid was measured in lipid nanoparticles prepared from them. The lipid nanoparticles were prepared as described in Example 35 using AD-1661 as the payload. The retention times are reported in the table below relative to the retention time for cholesterol.

The HPLC buffer used was a mixture of two solutions (Solution #1 and Solution #2).

Solution #1: 80% methanol / 20% 10 mM NH_4HCO_3

Solution #2: 80% methanol / 20% isopropanol

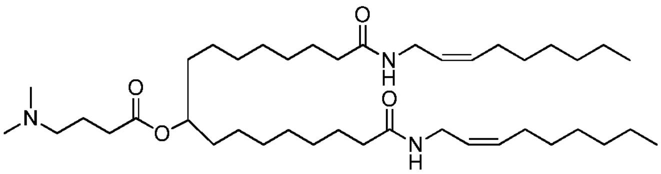
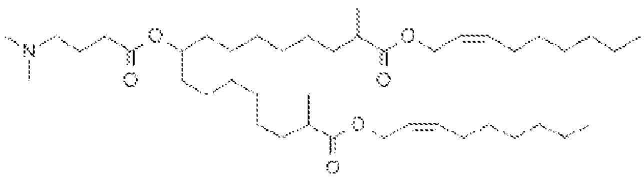
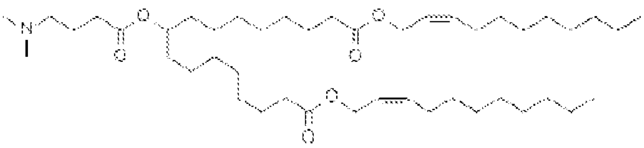
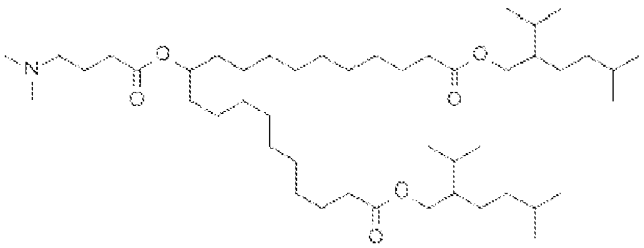
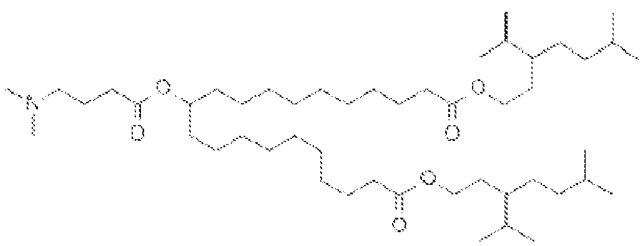
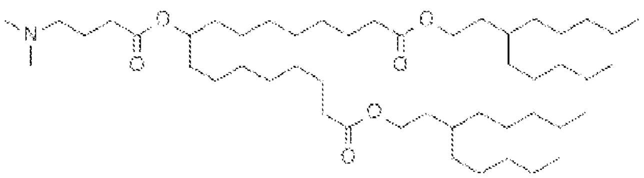
The ratios of the two solutions in the mixture changed over time as indicated in the table below.

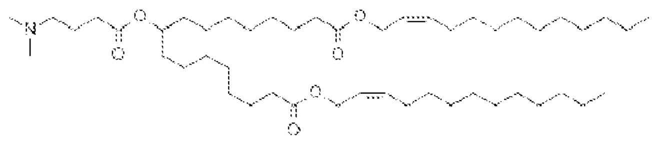
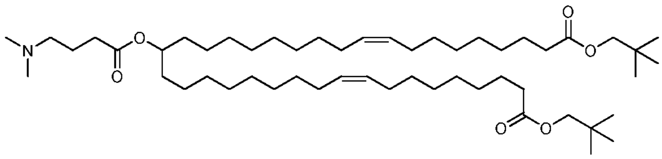
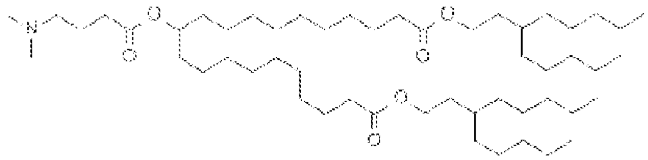
Time (min)	Solution #1 (vol%)	Solution #2 (vol%)
0	70	30
4	10	90
6	10	90
6.1	70	30
8	70	30

The size of the lipid nanoparticles was measured before and after undergoing dialysis overnight. In general, greater changes in lipid nanoparticle size are indicative of lesser stability.

Dynamic laser light scattering was used to determine the lipid nanoparticle size (expressed as the intensity weighted diameter) with a Zetasizer (Malvern Instruments, Inc. of Westborough, MA). All measurements were made at 532 nm wavelength at the scattering angle of 173° using normal resolution mode as the analysis model.

The results of these experiments are provided in the table below.

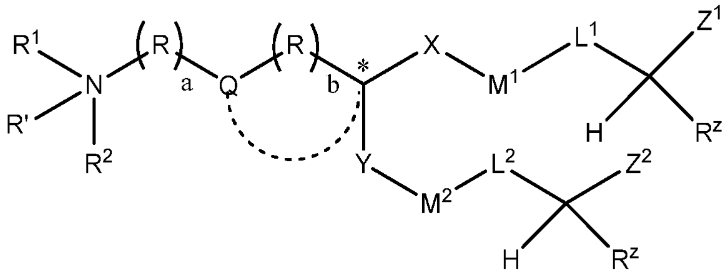
Cationic Lipid	logP	t(lipid) - t(chol)	LNPs Size (nm) change
	9.647	-1.4	170 -> 260
	9.972	0.848	73 -> 77
	10.093	1.44	60 -> 67
	10.201	1.751	59 -> 60
	10.259	2.106	
	10.313	2.365	56 -> 56

	10.315	2.219	68 -> 67
	10.416	2.707	
	10.495	3.178	

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

WHAT IS CLAIMED IS:

1. A compound of the formula:



Formula (III)

or a salt thereof, wherein

R' is absent, hydrogen, or C_1-C_4 alkyl;

with respect to R^1 and R^2,

(i) R^1 and R^2 are each, independently, optionally substituted alkyl, alkenyl, alkynyl, cycloalkylalkyl, heterocycle, or R^{10};

(ii) R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring; or

(iii) one of R^1 and R^2 is optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, or heterocycle, and the other forms a 4-10 member heterocyclic ring or heteroaryl with (a) the adjacent nitrogen atom and (b) the $(R)_a$ group adjacent to the nitrogen atom;

each occurrence of R is, independently, $-(CR^3R^4)-$;

each occurrence of R^3 and R^4 are, independently H, halogen, OH, alkyl, alkoxy, $-NH_2$, R^{10}, alkylamino, or dialkylamino;

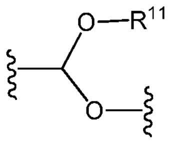
each occurrence of R^{10} is independently selected from PEG and polymers based on poly(oxazoline), poly(ethylene oxide), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), poly[N-(2-hydroxypropyl)methacrylamide] and poly(amino acid)s, wherein (i) the PEG or polymer is linear or branched, (ii) the PEG or polymer is polymerized by n subunits, (iii) n is a number-averaged degree of polymerization between 10 and 200 units, and (iv) wherein the compound of formula has at most two R^{10} groups;

the dashed line to Q is absent or a bond;

when the dashed line to Q is absent then Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)N(R^4)-, -N(R^5)C(O)-, -S-S-, -OC(O)O-, -O-N=C(R^5)-, -C(R^5)=N-O-, -OC(O)N(R^5)-, -N(R^5)C(O)N(R^5)-, -N(R^5)C(O)O-, -C(O)S-, -C(S)O- or -C(R^5)=N-O-C(O)-; or

when the dashed line to Q is a bond then (i) b is 0 and (ii) Q and the tertiary carbon adjacent to it (C*) form a substituted or unsubstituted, mono- or bi-cyclic heterocyclic group having from 5 to 10 ring atoms;

each occurrence of R^5 is, independently, H or C_1-C_4 alkyl;

M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or 

(wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

each occurrence of R^z is, independently, C_1-C_8 alkyl;

a is 1, 2, 3, 4, 5 or 6;

b is 0, 1, 2, or 3;

L^1 and L^2 are each, independently, C_1-C_5 alkylene or C_2-C_5 alkenylene;

X and Y are each, independently, C_4-C_{20} alkylene or C_4-C_{20} alkenylene; and

Z^1 and Z^2 are each, independently, C_8-C_{14} alkyl or C_8-C_{14} alkenyl, wherein the alkenyl group may optionally be substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of Z^1 or Z^2, and with the proviso that the terminus of at least one of Z^1 and Z^2 is separated from the group M^1 or M^2 by at least 8 carbon atoms.

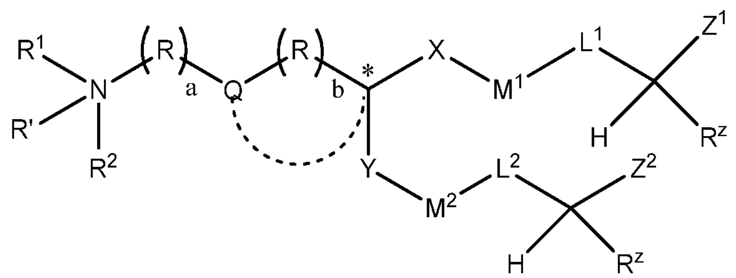
2. The compound of claim 1, wherein L^1 and L^2 are each $-CH_2-$.

3. The compound of claim 1, wherein L^1 and L^2 are each $-(CH_2)_2-$ or $-(CH_2)_3-$.

4. The compound of any of claims 1-3, wherein X and Y are each, independently $-(CH_2)_n$ wherein n is 4, 5, 6, 7 or 8.

5. The compound of claim 4, wherein X and Y are $-(CH_2)_7-$, $-(CH_2)_8-$, or $-(CH_2)_9-$.

6. A compound of the formula:



Formula (IIIA)

or a salt thereof, wherein

R' is absent, hydrogen, or C_1-C_4 alkyl;

with respect to R^1 and R^2,

(i) R^1 and R^2 are each, independently, optionally substituted alkyl, alkenyl, alkynyl, cycloalkylalkyl, heterocycle, or R^{10};

(ii) R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring; or

(iii) one of R^1 and R^2 is optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, or heterocycle, and the other forms a 4-10 member heterocyclic ring or heteroaryl with (a) the adjacent nitrogen atom and (b) the $(R)_a$ group adjacent to the nitrogen atom;

each occurrence of R is, independently, $–(CR^3R^4)$-;

each occurrence of R^3 and R^4 are, independently H, halogen, OH, alkyl, alkoxy, $-NH_2$, R^{10}, alkylamino, or dialkylamino;

each occurrence of R^{10} is independently selected from PEG and polymers based on poly(oxazoline), poly(ethylene oxide), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), poly[N-(2-hydroxypropyl)methacrylamide] and poly(amino acid)s, wherein (i) the PEG or polymer is linear or branched, (ii) the PEG or polymer is polymerized by n subunits, (iii) n is a number-averaged degree of polymerization between 10 and 200 units, and (iv) wherein the compound of formula has at most two R^{10} groups;

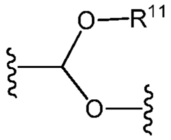
the dashed line to Q is absent or a bond;

when the dashed line to Q is absent then Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, $-C(O)N(R^4)$-, $-N(R^5)C(O)$-, -S-S-, -OC(O)O-, $-O-N=C(R^5)$-, $-C(R^5)=N-O$-, $-OC(O)N(R^5)$-, $-N(R^5)C(O)N(R^5)$-, $-N(R^5)C(O)O$-, -C(O)S-, -C(S)O- or $-C(R^5)=N-O-C(O)$-; or

when the dashed line to Q is a bond then (i) b is 0 and (ii) Q and the tertiary carbon adjacent to it (C*) form a substituted or unsubstituted, mono- or bi-cyclic heterocyclic group having from 5 to 10 ring atoms;

each occurrence of R^5 is, independently, H or C_1-C_4 alkyl;

M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, $-C(R^5)=N$-, $-N=C(R^5)$-, $-C(R^5)=N-O$-, $-O-N=C(R^5)$-, $-C(O)(NR^5)$-, $-N(R^5)C(O)$-, $-C(S)(NR^5)$-, $-N(R^5)C(O)$-, $-N(R^5)C(O)N(R^5)$-, –OC(O)O-, -

$OSi(R^5)_2O$-, $-C(O)(CR^3R^4)C(O)O$-, $-OC(O)(CR^3R^4)C(O)$-, or  (wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

each occurrence of R^z is, independently, C_1-C_8 alkyl;

a is 1, 2, 3, 4, 5 or 6;

b is 0, 1, 2, or 3;

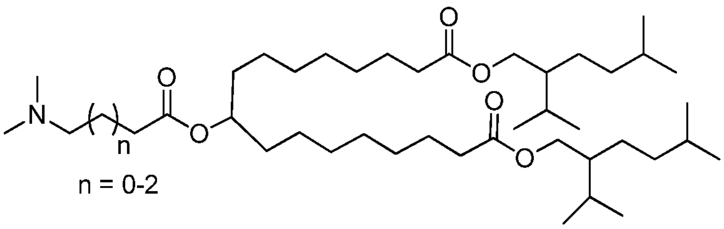
each R^z is, independently, C_1-C_8 alkyl;

L^1 and L^2 are each, independently, C_1-C_5 alkylene or C_2-C_5 alkenylene;

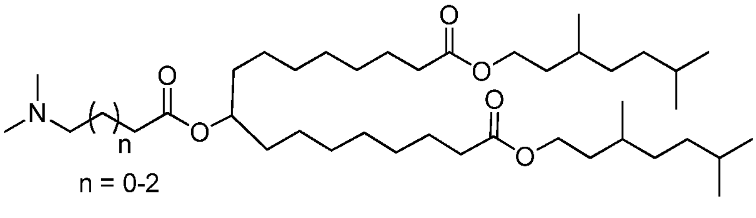
X and Y are each, independently, C_4-C_{20} alkylene or C_4-C_{20} alkenylene; and

Z^1 and Z^2 are each, independently, C_1-C_8 alkyl or C_2-C_8 alkenyl;

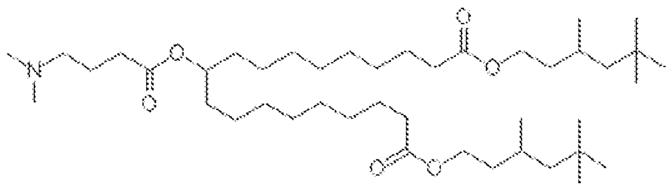
wherein said cationic lipid is not one selected from:

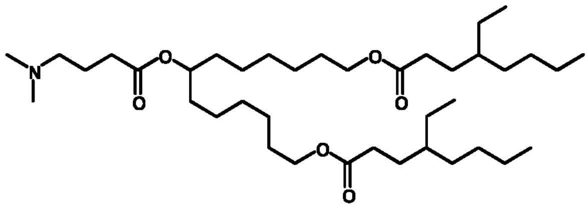


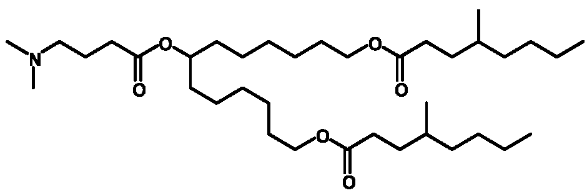
n = 0-2

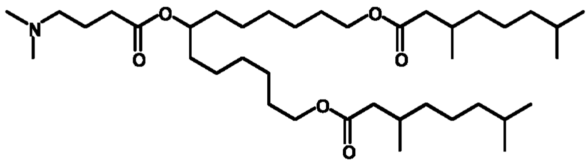


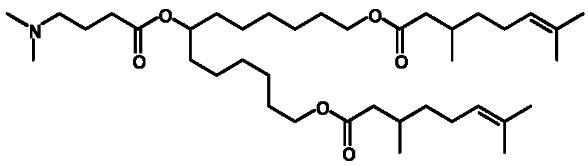
n = 0-2

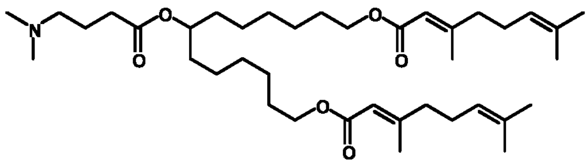












7. The compound of claim 6, wherein L^1 and L^2 are each -$(CH_2)_2$-.

8. The compound of claim 6, wherein L^1 and L^2 are each -$(CH_2)_3$-.

9. The compound of any one of claims 6-8, wherein X and Y are each, independently, –$(CH_2)_n$– wherein n is 7-9.

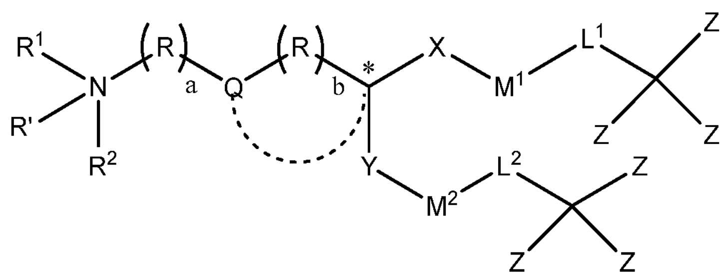
10. The compound of any one of claims 6-9, wherein M^1 and M^2 are –C(O)O- (where the carbonyl group in M^1 and M^2 is bound to the variable X, and the oxygen atom in M^1 and M^2 is bound to the variable L^1 and L^2).

11. The compound of any one of claims 6-10, wherein the R'R^1R^2N-$(R)_a$-Q-$(R)_b$- group is selected from $(CH_3)_2N$-$(CH_2)_3$-C(O)O-, $(CH_3)_2N$-$(CH_2)_2$-NH-C(O)O-, $(CH_3)_2N$-$(CH_2)_2$-OC(O)-NH-, and $(CH_3)_2N$-$(CH_2)_3$-$C(CH_3)$=N-O-.

12. The compound of any one of claims 6-11, wherein Z^1, Z^2, and each R^z are C_3-C_8 alkyl.

13. The compound of claim 12, wherein Z^1, Z^2, and each R^z are C_3-C_6 alkyl.

14. A compound of the formula:



Formula (IV)

or a salt thereof, wherein

R' is absent, hydrogen, or C_1-C_4 alkyl;

with respect to R^1 and R^2,

(i) R^1 and R^2 are each, independently, optionally substituted alkyl, alkenyl, alkynyl, cycloalkylalkyl, heterocycle, or R^{10};

(ii) R^1 and R^2, together with the nitrogen atom to which they are attached, form an optionally substituted heterocylic ring; or

(iii) one of R^1 and R^2 is optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, or heterocycle, and the other forms a 4-10 member heterocyclic ring or heteroaryl with (a) the adjacent nitrogen atom and (b) the $(R)_a$ group adjacent to the nitrogen atom;

each occurrence of R is, independently, –(CR^3R^4)-;

each occurrence of R^3 and R^4 are, independently H, halogen, OH, alkyl, alkoxy, -NH_2, R^{10}, alkylamino, or dialkylamino;

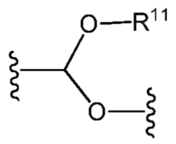
each occurrence of R^{10} is independently selected from PEG and polymers based on poly(oxazoline), poly(ethylene oxide), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), poly[N-(2-hydroxypropyl)methacrylamide] and poly(amino acid)s, wherein (i) the PEG or polymer is linear or branched, (ii) the PEG or polymer is polymerized by n subunits, (iii) n is a number-averaged degree of polymerization between 10 and 200 units, and (iv) wherein the compound of formula has at most two R^{10} groups;

the dashed line to Q is absent or a bond;

when the dashed line to Q is absent then Q is absent or is -O-, -NH-, -S-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)N(R^4)-, -N(R^5)C(O)-, -S-S-, -OC(O)O-, -O-N=C(R^5)-, -C(R^5)=N-O-, -OC(O)N(R^5)-, -N(R^5)C(O)N(R^5)-, -N(R^5)C(O)O-, -C(O)S-, -C(S)O- or -C(R^5)=N-O-C(O)-; or

when the dashed line to Q is a bond then (i) b is 0 and (ii) Q and the tertiary carbon adjacent to it (C*) form a substituted or unsubstituted, mono- or bi-cyclic heterocyclic group having from 5 to 10 ring atoms;

each occurrence of R^5 is, independently, H or C_1-C_4 alkyl;

M^1 and M^2 are each, independently, a biodegradable group (e.g., -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, -C(R^5)=N-, -N=C(R^5)-, -C(R^5)=N-O-, -O-N=C(R^5)-, -C(O)(NR^5)-, -N(R^5)C(O)-, -C(S)(NR^5)-, -N(R^5)C(O)-, -N(R^5)C(O)N(R^5)-, –OC(O)O-, -OSi(R^5)$_2$O-, -C(O)(CR^3R^4)C(O)O-, -OC(O)(CR^3R^4)C(O)-, or 

(wherein R^{11} is a C_2-C_8 alkyl or alkenyl));

each occurrence of R^z is, independently, C_1-C_8 alkyl;

a is 1, 2, 3, 4, 5 or 6;

b is 0, 1, 2, or 3;

L^1 and L^2 and are each, independently, C_1-C_5 alkylene or C_2-C_5 alkenylene;

X and Y are each, independently, alkylene or alkenylene; and

each occurrence of Z is independently C_1-C_4 alkyl (preferably, methyl).

15. The compound of claim 14, wherein X and Y are C_{12}-C_{20} alkylene or C_{12}-C_{20} alkenylene.

16. The compound of claim 14 or 15, wherein -L^1-$C(Z)_3$ is –$CH_2C(CH_3)_3$ or –$CH_2CH_2C(CH_3)_3$.

17. A cationic lipid or a salt thereof having:
(i) a central carbon atom,

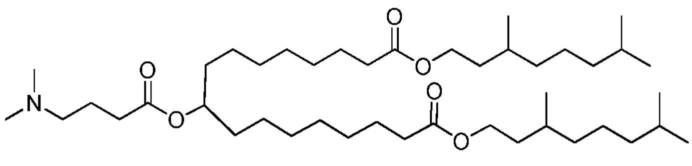
(ii) a nitrogen containing head group directly bound to the central carbon atom, and

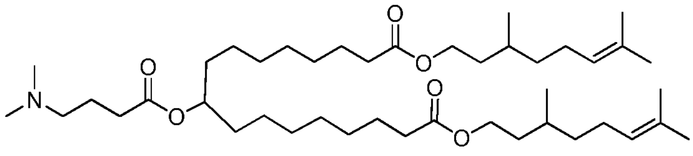
(iii) two hydrophobic tails directly bound to the central carbon atom, wherein each hydrophobic tail is of the formula –R^e-M-R^f where R^e is a C_4-C_{14} alkyl or alkenyl, M is a biodegradable group, and R^f is a branched alkyl or alkenyl, such that (i) the chain length of –R^e-M-R^f is at most 20 atoms, and (ii) the group –R^e-M-R^f has at least 20 carbon atoms, wherein

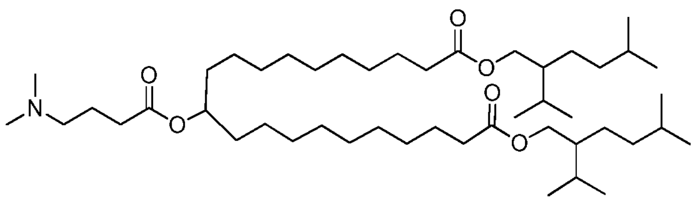
the alkyl or alkenyl group in R^e is optionally substituted with one or two fluorine atoms at the alpha position to the M^1 or M^2 group; and

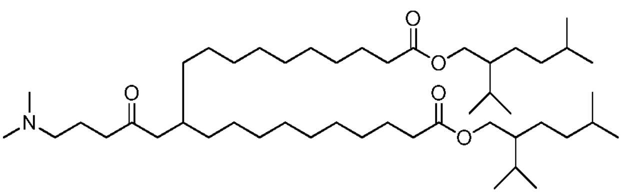
the alkenyl group in R^f is optionally substituted with one or two fluorine atoms at the alpha position to a double bond which is between the double bond and the terminus of R^f.

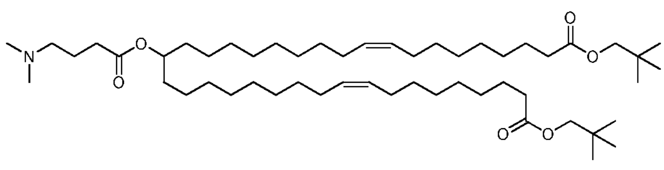
18. A compound selected from:

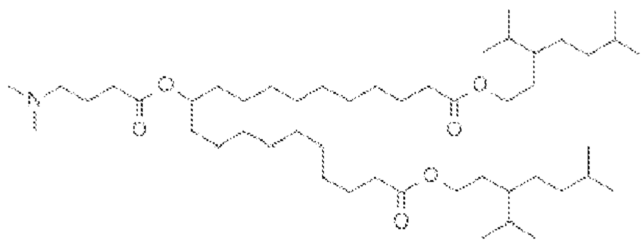


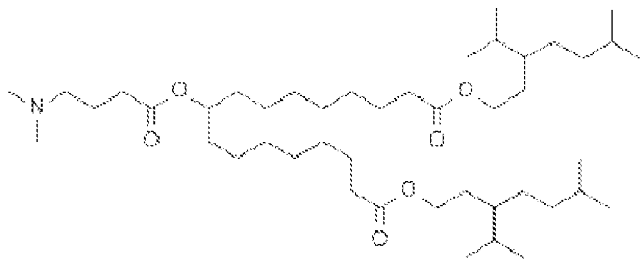


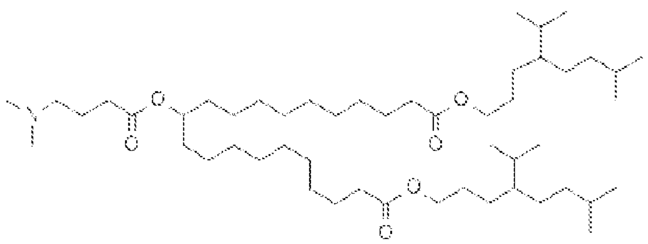


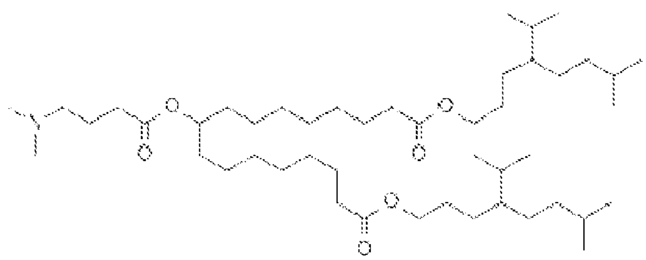


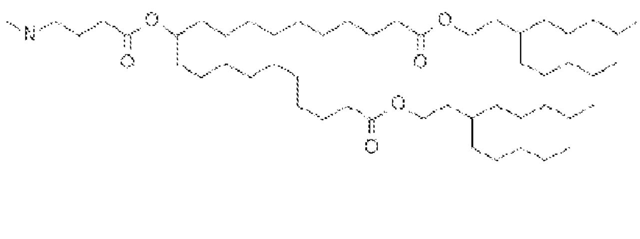


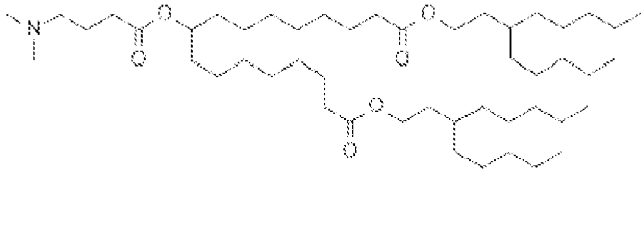


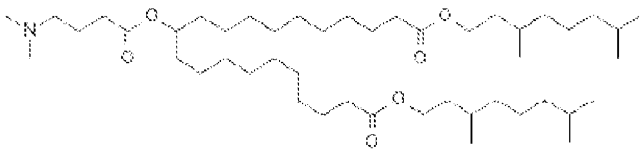


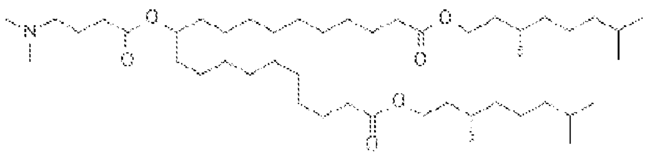






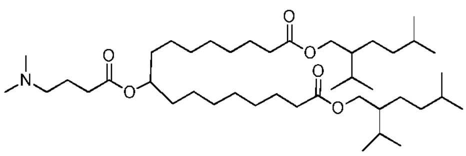


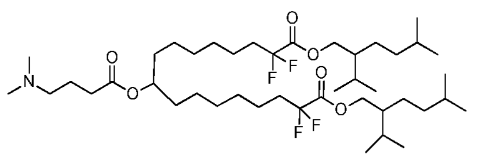


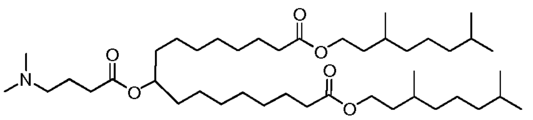


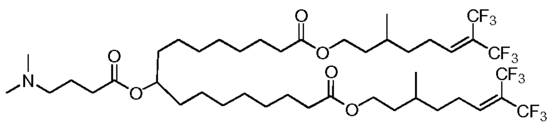
and salts thereof.

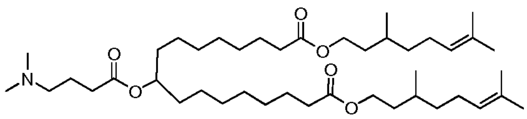
19. A compound selected from:

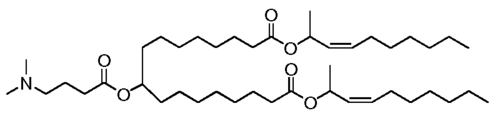
 ,

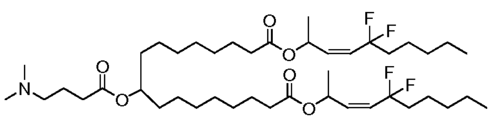
 ,

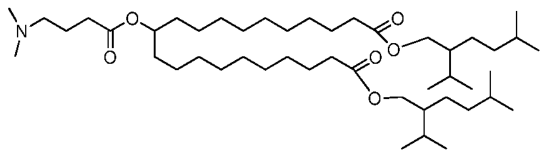
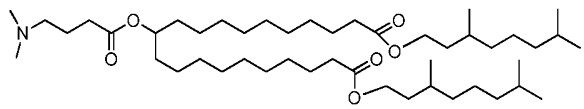
 ,

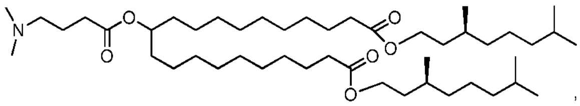
 ,

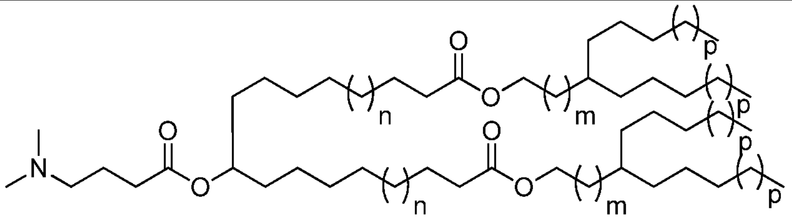
 ,

 ,

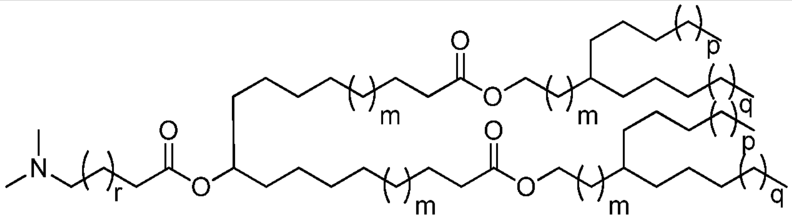
 ,

,
,

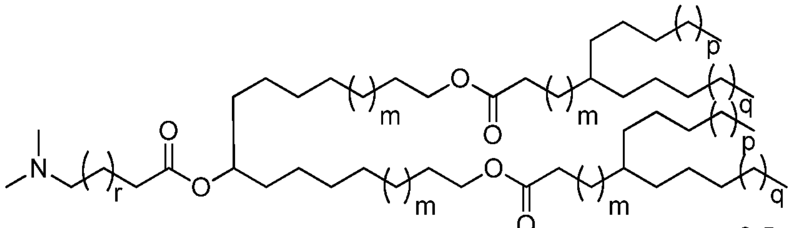
,



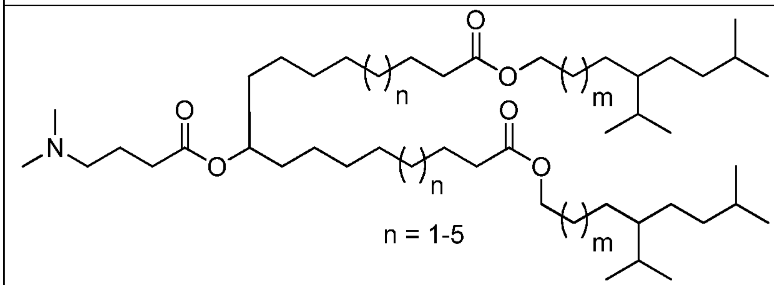
n = 1-5 m = 0-3 p = 1-5



r = 0-2 n = 0-5 m = 0-3 p = 0-5 q = 0-5

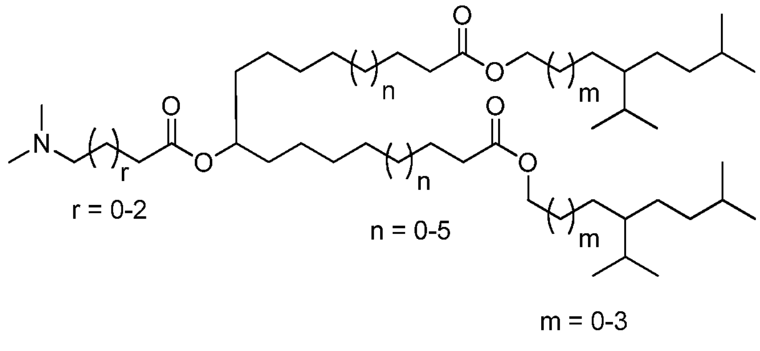


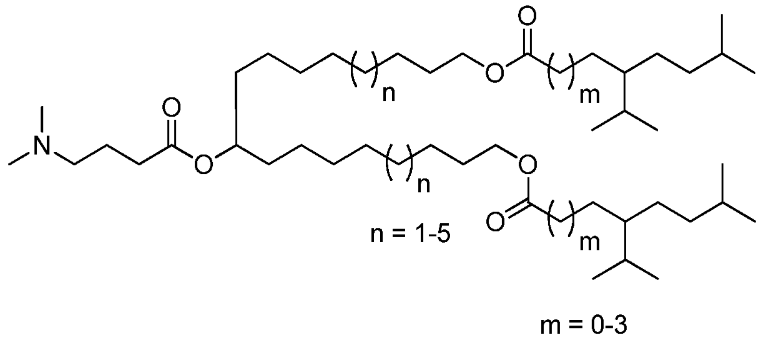
r = 0-2 n = 0-5 m = 0-3 p = 0-5 q = 0-5

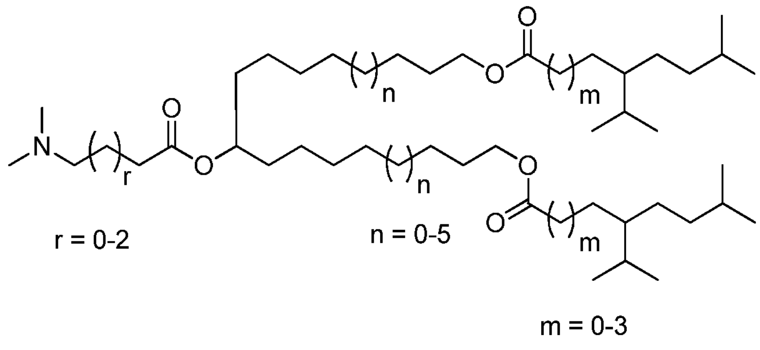


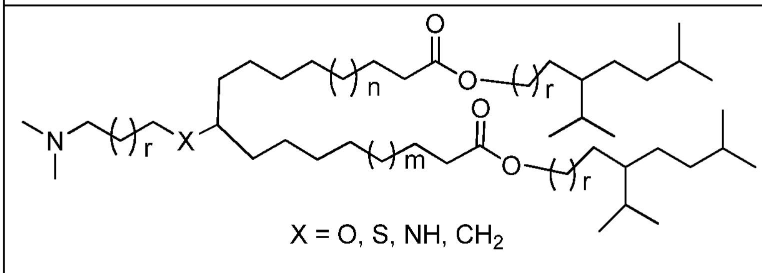
n = 1-5

m = 0-3

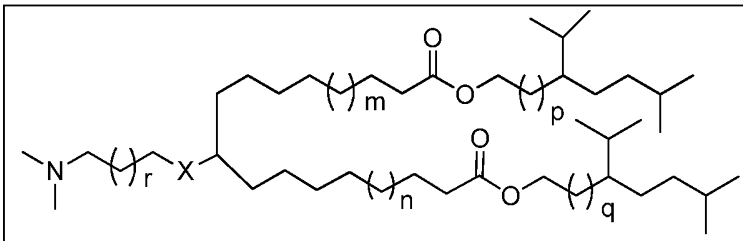




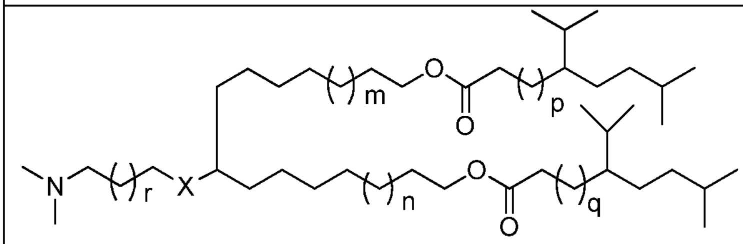




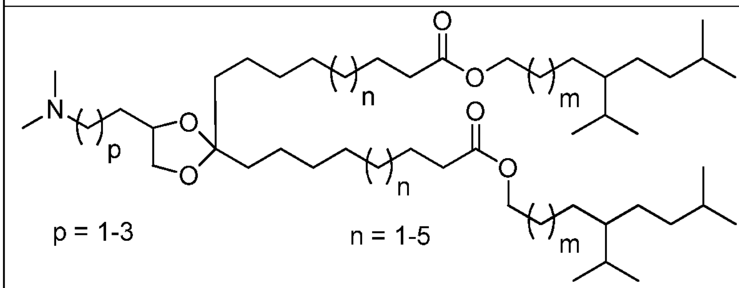
r = 0-2, n = 1-5, and m = 1-5



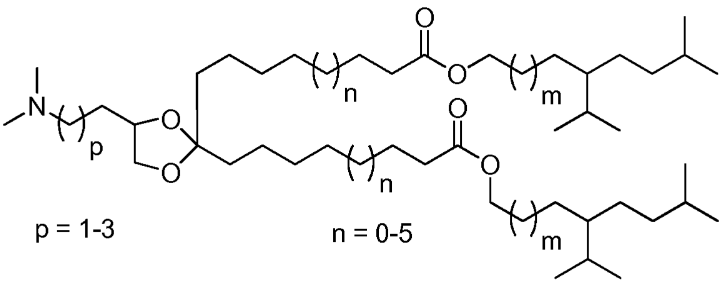
r = 0-2 X = O, S, NH, CH_2 m = 0-5 n = 0-5 p = 0-3 q = 0-3



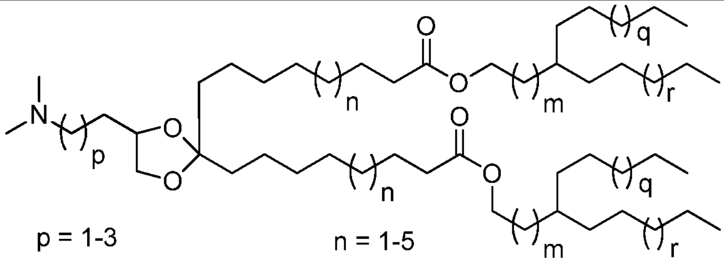
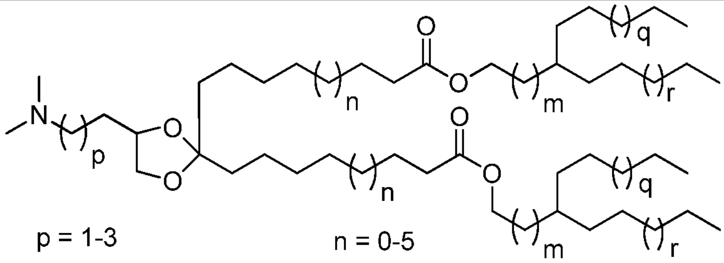
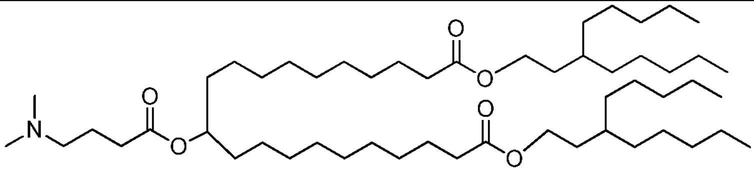
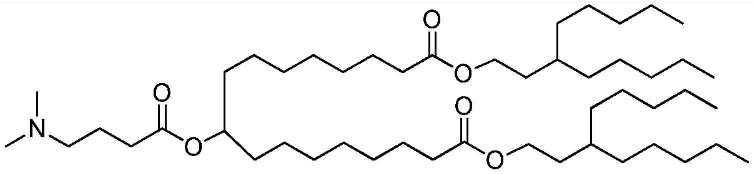
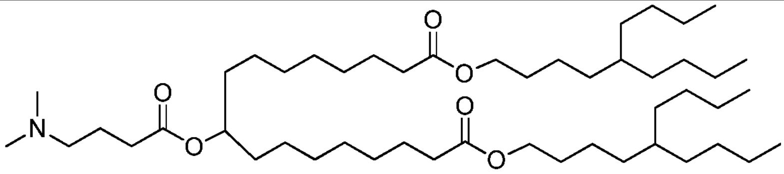
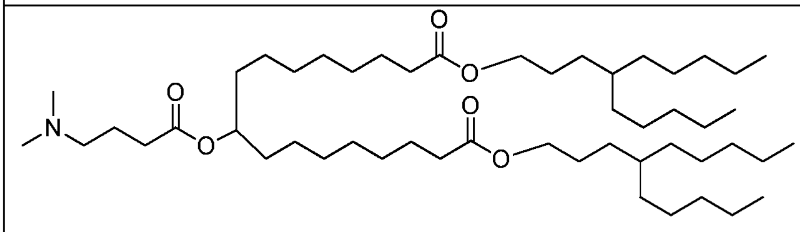
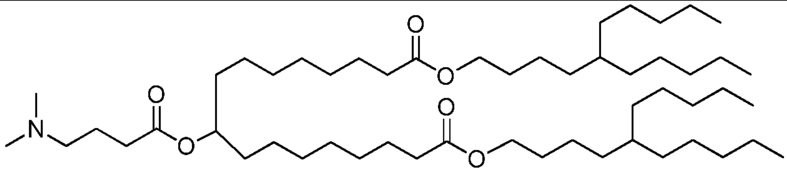
r = 0-2 X = O, S, NH, CH_2 m = 0-5 n = 0-5 p = 0-3 q = 0-3

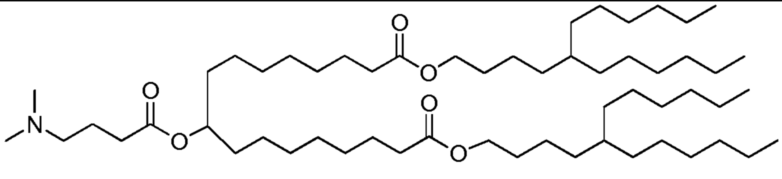


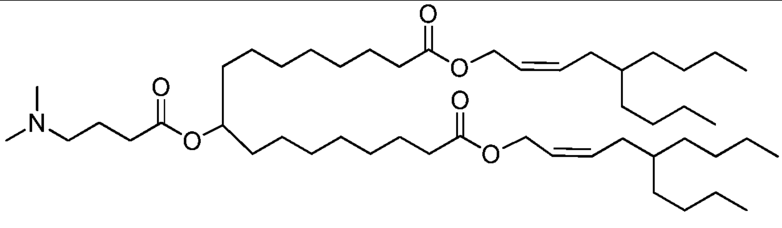
p = 1-3 n = 1-5 m = 0-3

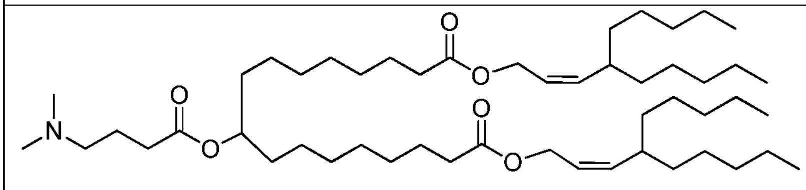


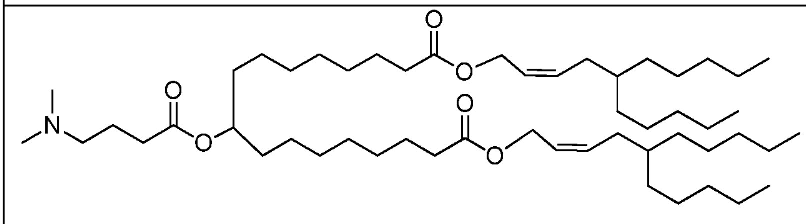
p = 1-3 n = 0-5 m = 0-3

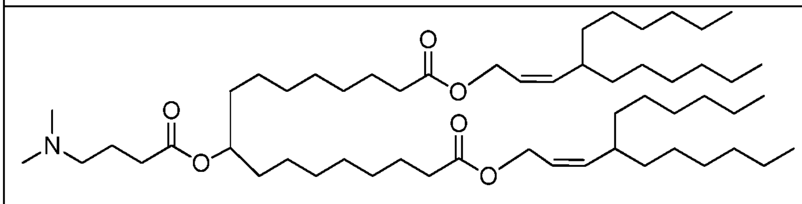
 p = 1-3 n = 1-5 m = 0-3; q = 0-4; r = 0-4
 p = 1-3 n = 0-5 m = 0-3; q = 0-4; r = 0-4






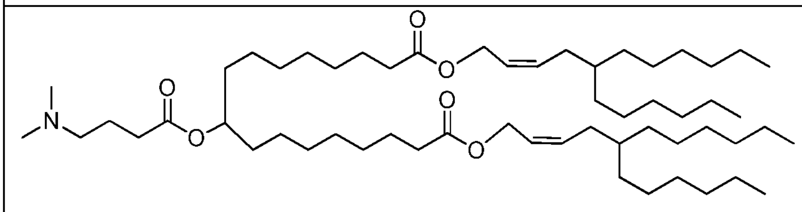


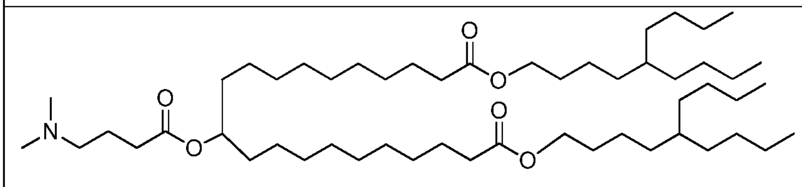


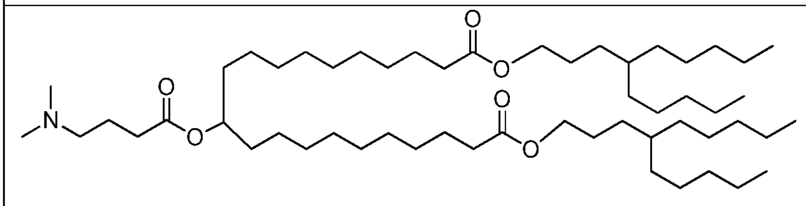


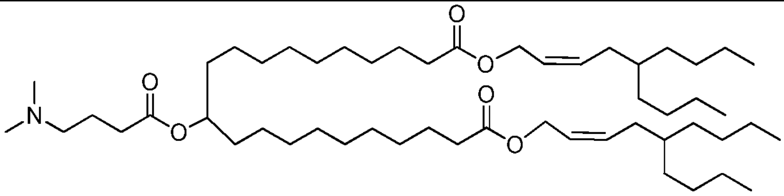
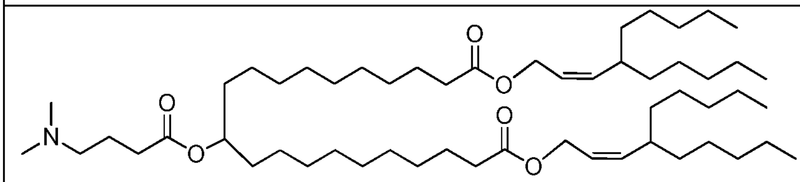
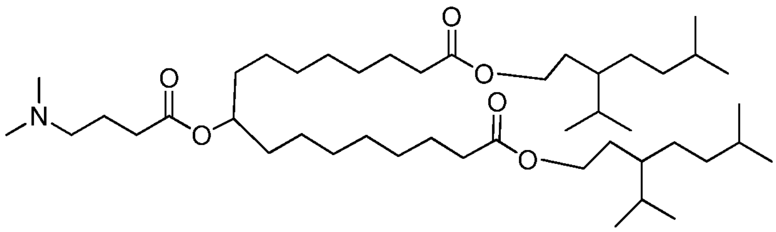
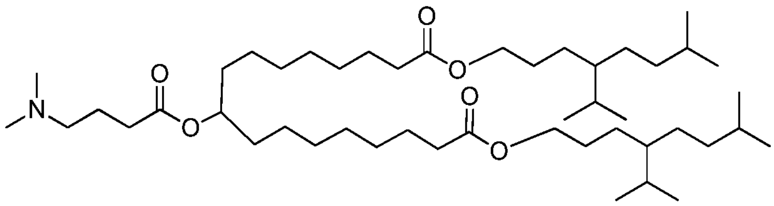
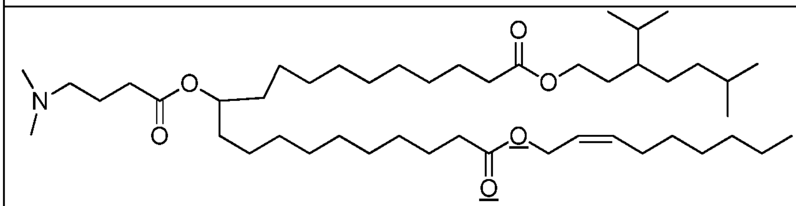
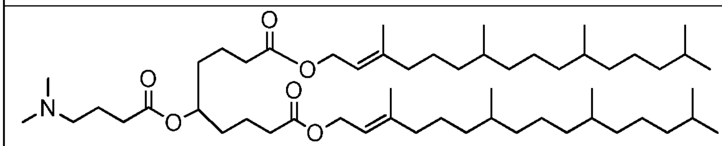
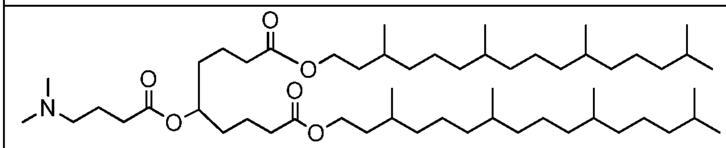
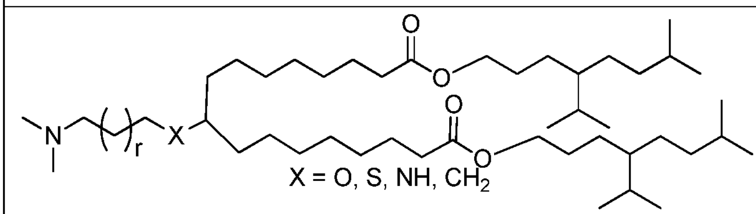


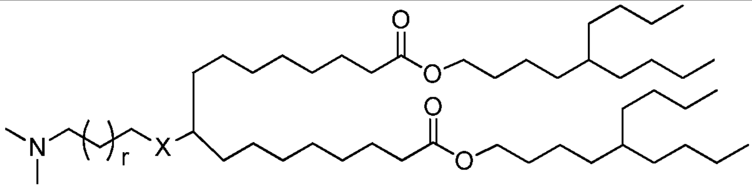
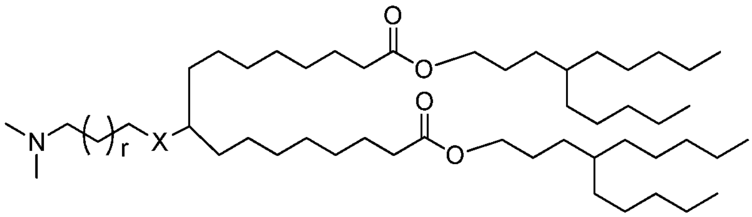
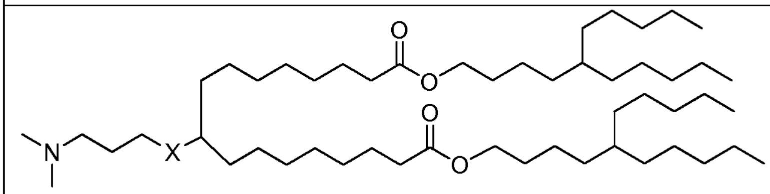
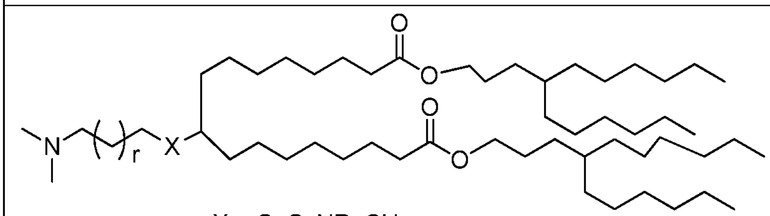
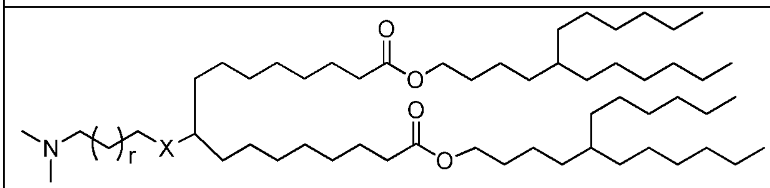


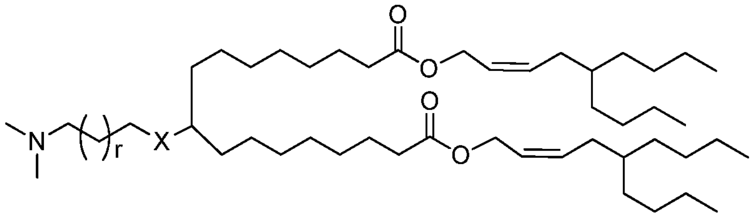
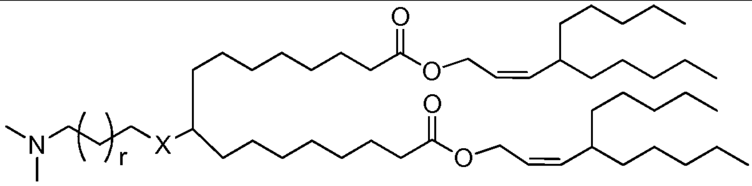
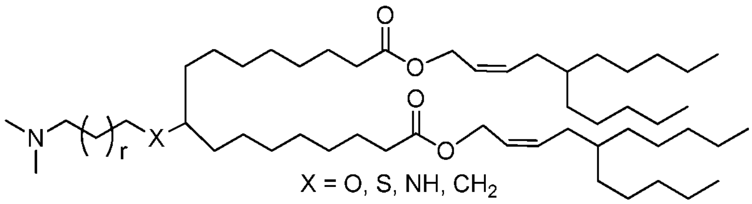
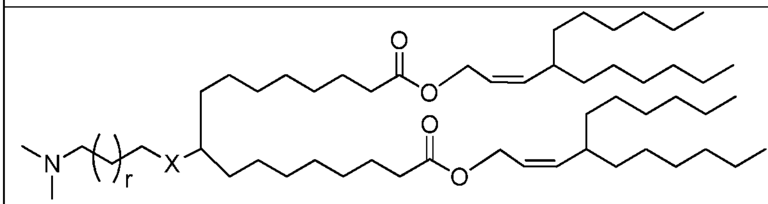
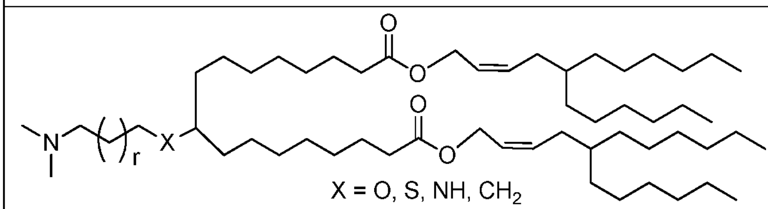


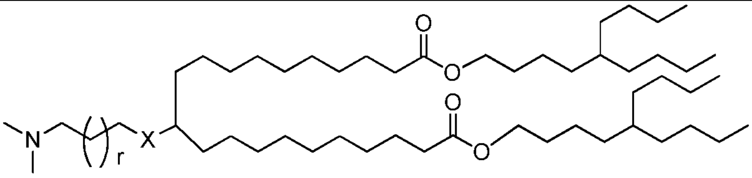
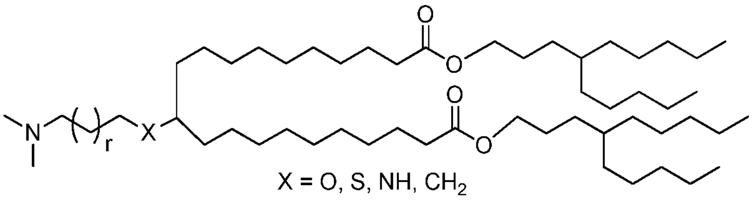
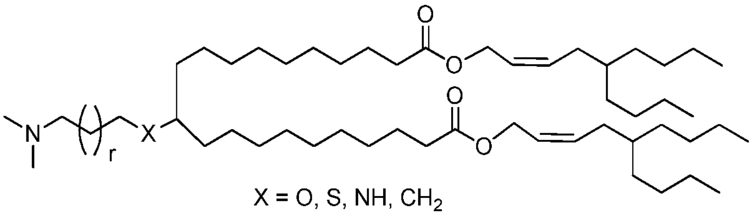
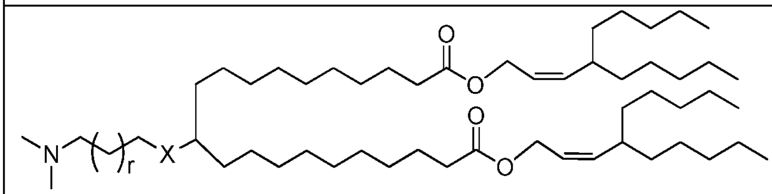
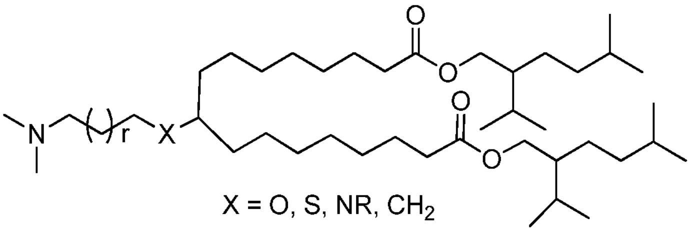


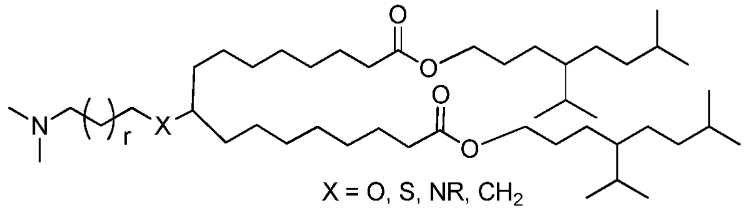









X = O, S, NH, CH2

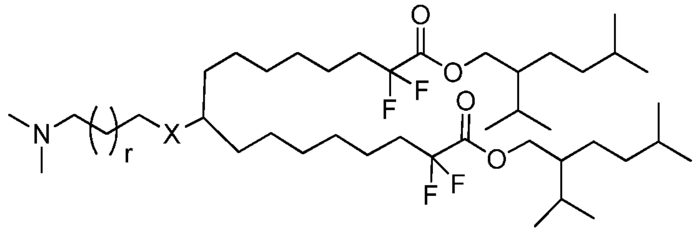
r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2
 X = O, S, NR, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2

 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2

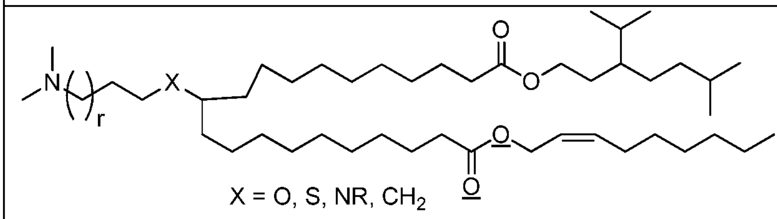
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NH, CH_2 r = 0, 1, or 2
 X = O, S, NR, CH_2 r = 0, 1, or 2



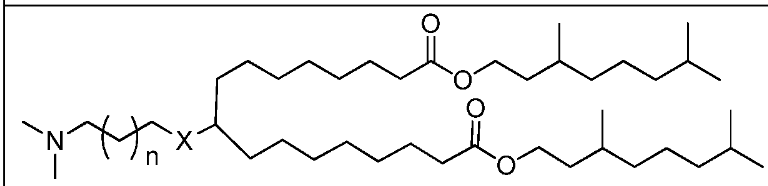
r = 0, 1, or 2



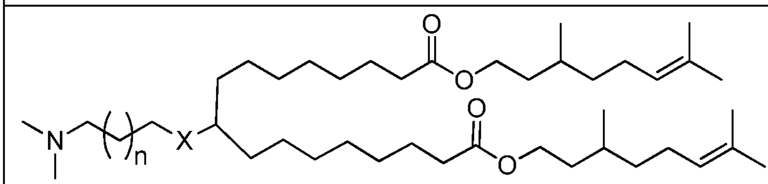
r = 0, 1, or 2



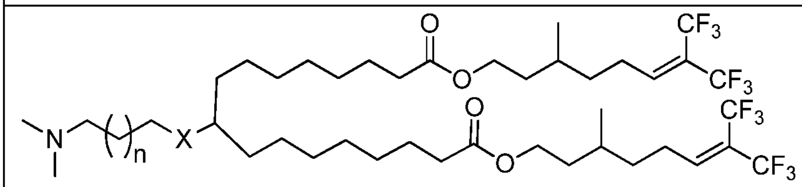
r = 0, 1, or 2



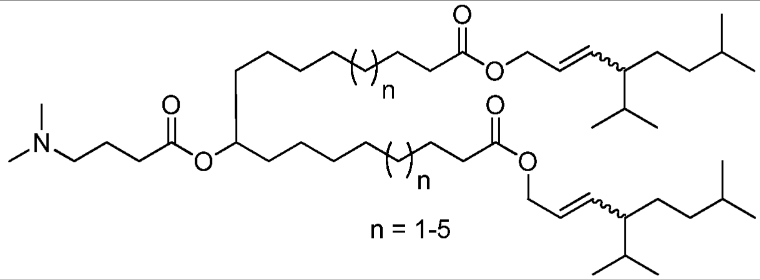
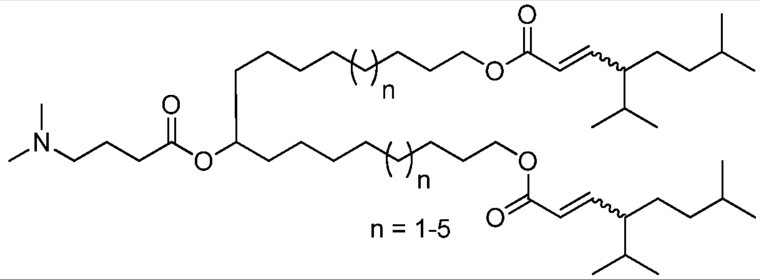
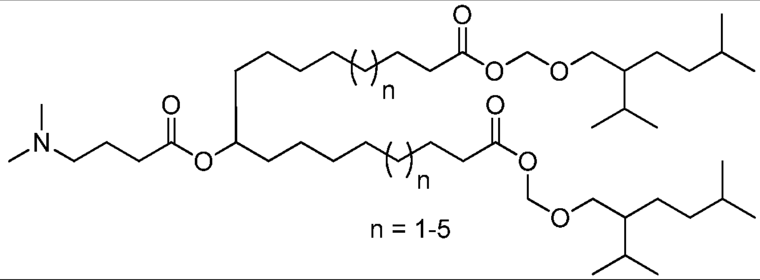
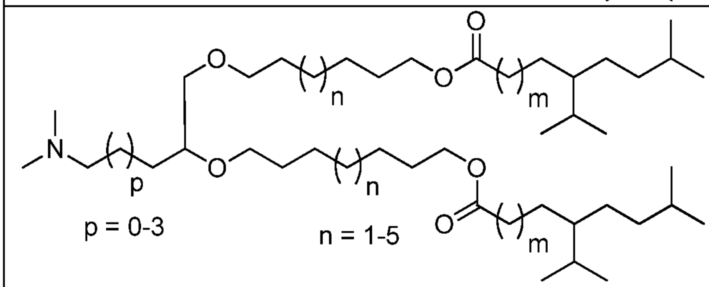
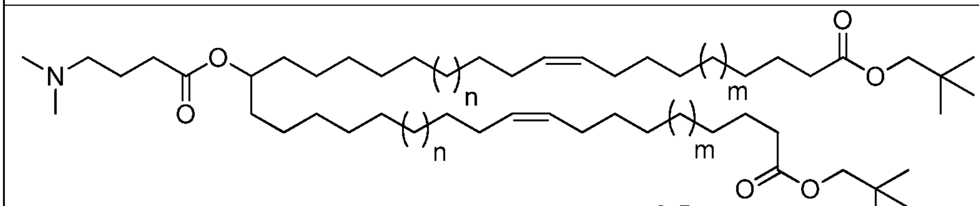
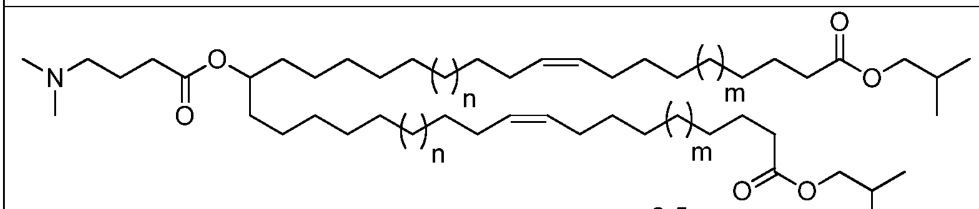
n = 0, 1, or 2

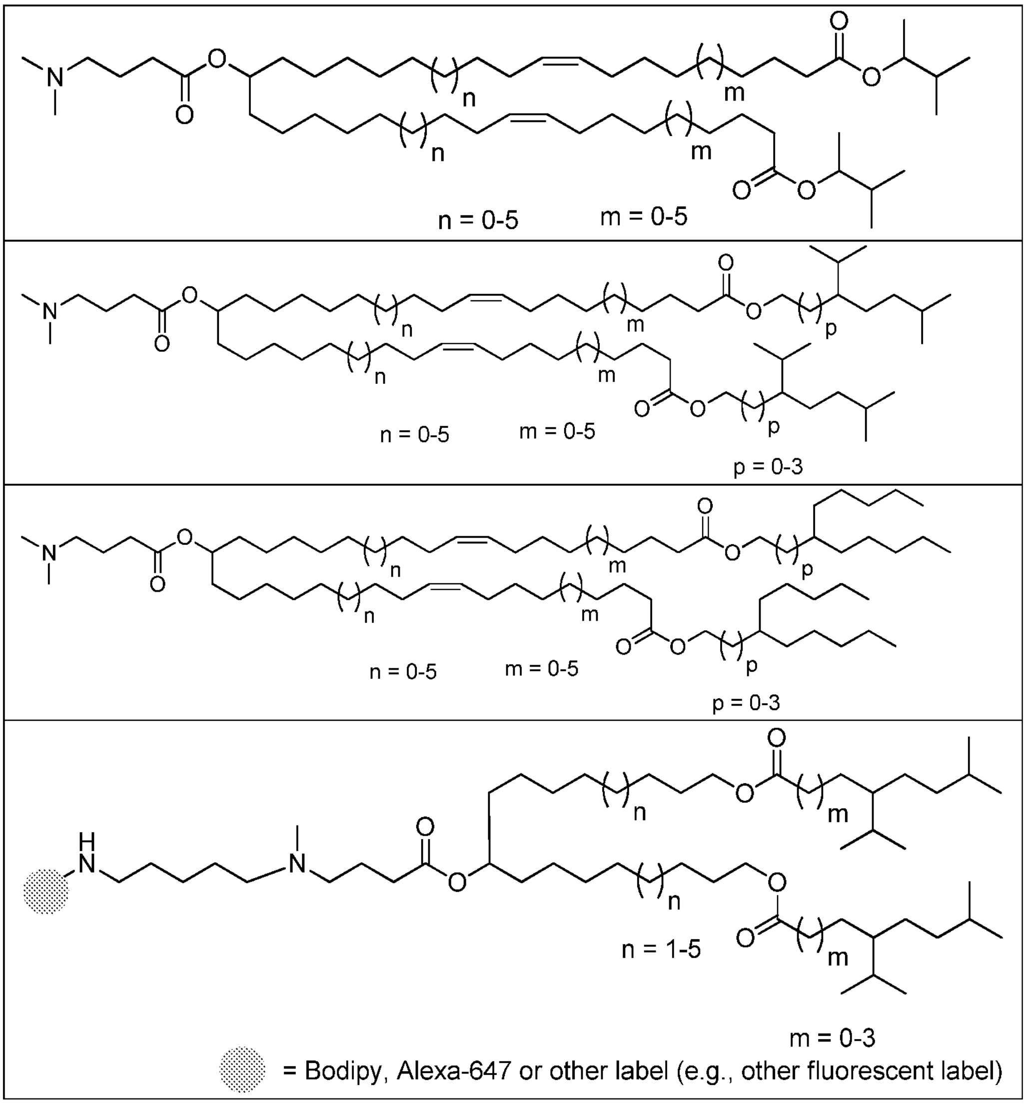


n = 0, 1, or 2



n = 0, 1, or 2

 n = 1-5
 n = 1-5
 n = 1-5
 p = 0-3 n = 1-5 m = 0-3
 n = 0-5 m = 0-5
 n = 0-5 m = 0-5



and salts thereof.

20. The compound of any of the preceding claims, wherein the compound is in the form of a pharmaceutically acceptable salt.

21. The compound of any of the preceding claims, wherein the compound is in the form of a cationic lipid.

22. A lipid particle comprising a neutral lipid, a lipid capable of reducing aggregation, and a cationic lipid of claim 21.

23. The lipid particle of claim 22, wherein the neutral lipid is selected from DSPC, DPPC, POPC, DOPE, or SM; the lipid capable of reducing aggregation is a PEG lipid; and the lipid particle further comprises a sterol.

24. The lipid particle of any one of claims 22 and 23, wherein the cationic lipid is present in a mole percentage of about 20% and about 60%; the neutral lipid is present in a mole percentage of about 5% to about 25%; the sterol is present in a mole percentage of about 25% to about 55%; and the PEG lipid is PEG-DMA, PEG-DMG, or a combination thereof, and is present in a mole percentage of about 0.5% to about 15%.

25. The lipid particle of claim 22, wherein the lipid capable of reducing aggregation is PEG-DMG.

26. The lipid particle of claim 25, wherein the lipid particle comprises about 50 mole % of the cationic lipid, about 10% DSPC, about 38.5% cholesterol, and about 1.5% PEG-DMG (based on 100% of the lipid components in the lipid particle).

27. The lipid particle of any of claims 22-26, further comprising an active agent.

28. The lipid particle of claim 27, wherein the active agent is a nucleic acid selected from a plasmid, an immunostimulatory oligonucleotide, an siRNA, an antisense oligonucleotide, a microRNA, an antagomir, an aptamer, and a ribozyme.

29. A pharmaceutical composition comprising a lipid particle of any one of claims 22-28 and a pharmaceutically acceptable carrier.

30. A method of modulating the expression of a target gene in a cell, comprising providing to the cell a lipid particle of any one of claims 22-28.

31. The method of claim 30, wherein the active agent is a nucleic acid is an siRNA.

32. A method of treating a disease or disorder characterized by the overexpression of a polypeptide in a subject, comprising providing to the subject the pharmaceutical composition of claim 29, wherein the active agent is a nucleic acid selected from the group consisting of an siRNA, a microRNA, and an antisense oligonucleotide, and wherein the siRNA, microRNA, or antisense oligonucleotide includes a polynucleotide that specifically binds to a polynucleotide that encodes the polypeptide, or a complement thereof.

33. A method of treating a disease or disorder characterized by underexpression of a polypeptide in a subject, comprising providing to the subject the pharmaceutical composition of claim 29, wherein the active agent is a plasmid that encodes the polypeptide or a functional variant or fragment thereof.

34. A method of inducing an immune response in a subject, comprising providing to the subject the pharmaceutical composition of claim 29, wherein the active agent is an immunostimulatory oligonucleotide.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/068491

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/713 A61K31/7088 C07F5/02 C07C229/12 C07C327/22
C07C327/32 C07C211/09 C07C211/10 C07C211/11 C07C217/08
C07C251/38 C07C327/28 C07D317/30 C07C235/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C C07D C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/141704 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; HEYES JAMES [CA]; WOOD MARK [CA]; MA) 17 November 2011 (2011-11-17) examples 1-13 -----	1-34
X	WO 2011/141705 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; HEYES JAMES [CA]; WOOD MARK [CA]; MA) 17 November 2011 (2011-11-17) claims 1-58; examples 1-9, 13-19, 25-27, 29 -----	1-34
X	WO 2010/054401 A1 (ALNYLAM PHARMACEUTICALS INC [US]; MANOHARAN MUTHIAH [US]; JAYARAMAN MU) 14 May 2010 (2010-05-14) claims 1-25; examples 17, 18, 20-30, 32-41 -----	1-34
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2013	05/04/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheid, Günther

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHESNOY S ET AL: "Structure and function of lipid-DNA complexes for gene delivery", ANNUAL REVIEW OF BIOPHYSICS AND BIOMOLECULAR STRUCTURE, ANNUAL REVIEWS INC., PALO ALTO, CA, US, vol. 29, 1 January 2000 (2000-01-01), pages 27-47, XP002664604, ISSN: 1056-8700 figures 2, 3 -----	1-34
X,P	WO 2011/153493 A2 (ALNYLAM PHARMACEUTICALS INC [US]; MANOHARAN MUTHIAH [US]; MAIER MARTIN) 8 December 2011 (2011-12-08) cited in the application claims 1-55; examples 7, 8, 9 -----	1-34

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/068491

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011141704 A1	17-11-2011	NONE	
WO 2011141705 A1	17-11-2011	CA 2799091 A1	17-11-2011
		EP 2569276 A1	20-03-2013
		WO 2011141705 A1	17-11-2011
WO 2010054401 A1	14-05-2010	AU 2009313201 A1	14-05-2010
		AU 2009313205 A1	14-05-2010
		AU 2009313206 A1	14-05-2010
		CA 2743135 A1	14-05-2010
		CA 2743136 A1	14-05-2010
		CA 2743139 A1	14-05-2010
		CN 102281899 A	14-12-2011
		CN 102361650 A	22-02-2012
		EA 201170553 A1	30-05-2012
		EA 201170554 A1	30-04-2012
		EP 2355658 A1	17-08-2011
		EP 2355851 A1	17-08-2011
		EP 2367571 A1	28-09-2011
		JP 2012508261 A	05-04-2012
		JP 2012508263 A	05-04-2012
		JP 2012508264 A	05-04-2012
		KR 20110091866 A	16-08-2011
		KR 20110097823 A	31-08-2011
		US 2011311582 A1	22-12-2011
		US 2011311583 A1	22-12-2011
		US 2012027796 A1	02-02-2012
		US 2012095075 A1	19-04-2012
		WO 2010054401 A1	14-05-2010
		WO 2010054405 A1	14-05-2010
		WO 2010054406 A1	14-05-2010
WO 2011153493 A2	08-12-2011	AU 2011261247 A1	20-12-2012
		CA 2800401 A1	08-12-2011
		EP 2575764 A2	10-04-2013
		US 2012027803 A1	02-02-2012
		WO 2011153493 A2	08-12-2011

摘要

本发明涉及一种阳离子脂质，其具有一个或多个位于该阳离子脂质的脂质成分（例如，一亲水链）中的可生物降解的团。这些阳离子脂质可被合并进脂质粒以递送一种活性剂，例如核酸。本发明还涉及脂质粒，其包含中性脂肪、能够减少聚合的脂质、本发明的阳离子脂质以及可选择包含固醇。所述脂质粒可进一步包括例如核酸的治疗剂。