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(54) **Title**: NOVEL LOW MOLECULAR WEIGHT, BIODEGRADABLE CATIONIC LIPIDS FOR OLIGONUCLEOTIDE DELIVERY

(57) **Abstract**: The instant invention provides for novel cationic lipids that can be used in combination with other lipid components such as cholesterol and PEG-lipids to form lipid nanoparticles with oligonucleotides. It is an object of the instant invention to provide a cationic lipid scaffold that demonstrates enhanced efficacy along with lower liver toxicity as a result of lower lipid levels in the liver. The present invention employs low molecular weight cationic lipids with one short lipid chain coupled with inclusion of hydrolysable functionality in the lipid chains to enhance the efficiency and tolerability of in vivo delivery of siRNA.

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TITLE OF THE INVENTION

NOVEL LOW MOLECULAR WEIGHT, BIODEGRADABLE CATIONIC LIPIDS FOR OLIGONUCLEOTIDE DELIVERY

BACKGROUND OF THE INVENTION

The present invention relates to novel cationic lipids that can be used in combination with other lipid components such as cholesterol and PEG-lipids to form lipid nanoparticles with oligonucleotides, to facilitate the cellular uptake and endosomal escape, and to knockdown target mRNA both *in vitro* and *in vivo*.

Cationic lipids and the use of cationic lipids in lipid nanoparticles for the delivery of oligonucleotides, in particular siRNA and miRNA, have been previously disclosed. Lipid nanoparticles and use of lipid nanoparticles for the delivery of oligonucleotides, in particular siRNA and miRNA, has been previously disclosed. Oligonucleotides (including siRNA and miRNA) and the synthesis of oligonucleotides has been previously disclosed. (See US patent applications: US 2006/0083780, US 2006/0240554, US 2008/0020058, US 2009/0263407 and US 2009/0285881 and PCT patent applications: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405, WO2010/054406 and WO2011/153493). See also Semple S. C. et al., Rational design of cationic lipids for siRNA delivery, *Nature Biotechnology*, **2010**, *28*, 172-176.

Other cationic lipids are disclosed in the following patent applications: US 2009/0263407, US 2009/0285881, US 2010/0055168, US 2010/0055169, US 2010/0063135, US 2010/0076055, US 2010/0099738, US 2010/0104629, WO2010/088537, WO2010/144740, US2010/0324120, US 8,034,376, WO2011/143230, WO2011/000106, US2011/0117125, US2011/0256175, WO2011/141703, WO2011/141704 and WO2011/141705.

Traditional cationic lipids such as CLinDMA and DLinDMA have been employed for siRNA delivery to the liver but suffer from non-optimal delivery efficiency along with liver toxicity at higher doses. It is an object of the instant invention to provide a cationic lipid scaffold that demonstrates enhanced efficacy along with lower liver toxicity as a result of lower lipid levels in the liver. The present invention employs low molecular weight cationic lipids with one short lipid chain coupled with inclusion of hydrolysable functionality in the lipid chains to enhance the efficiency and tolerability of in vivo delivery of siRNA.

SUMMARY OF THE INVENTION

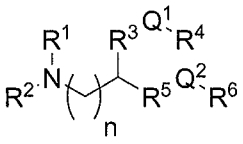
The instant invention provides for novel cationic lipids that can be used in combination with other lipid components such as cholesterol and PEG-lipids to form lipid nanoparticles with oligonucleotides. It is an object of the instant invention to provide a cationic lipid scaffold that demonstrates enhanced efficacy along with lower liver toxicity as a result of lower lipid levels in the liver. The present invention employs low molecular weight cationic lipids with one short lipid chain coupled with inclusion of hydrolysable functionality in the lipid chains to enhance the efficiency and tolerability of in vivo delivery of siRNA.

DETAILED DESCRIPTION OF THE INVENTION

The various aspects and embodiments of the invention are directed to the utility of novel cationic lipids useful in lipid nanoparticles to deliver oligonucleotides, in particular, siRNA and miRNA, to any target gene. (See US patent applications: US 2006/0083780, US 2006/0240554, US 2008/0020058, US 2009/0263407 and US 2009/0285881 and PCT patent applications: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405, WO2010/054406 and WO2011/153493). See also Semple S. C. et al., Rational design of cationic lipids for siRNA delivery, *Nature Biotechnology*, **2010**, *28*, 172-176.

The cationic lipids of the instant invention are useful components in a lipid nanoparticle for the delivery of oligonucleotides, specifically siRNA and miRNA.

In a first embodiment of this invention, the cationic lipids are illustrated by the Formula A:



A

wherein:

R^1 and R^2 are independently selected from H, $(C_1\text{-}C_6)$alkyl, heterocyclyl, and polyamine, wherein said alkyl, heterocyclyl and polyamine are optionally substituted with one to three substituents selected from R', or R^1 and R^2 can be taken together with the nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O

and S, said monocyclic heterocycle is optionally substituted with one to three substituents selected from R';

R^3 is independently selected from (C_4-C_{20})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from (C_1-C_{16})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from (C_4-C_8) alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from (C_1-C_2)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond, -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -S-S-, -C(R")=N-, -N=C(R")-, -C(R")=N-O-, -O-N=C(R")-, -C(O)(NR")-, -N(R")C(O)-, C(S)(NR")-, -N(R")C(O)-, -N(R")C(O)N(R")-, -OC(O)O-, $OSi(R")_2O$-, -C(O)($CR"_2$)C(O)O-, or –OC(O)($CR"_2$)C(O)-), with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

R' is independently selected from halogen, R", OR", SR", CN, $CO_2R"$ or $CON(R")_2$;

R" is independently selected from H and (C_1-C_6)alkyl, wherein said alkyl is optionally substituted with halogen and OH;

n is 0, 1, 2, 3, 4 or 5;

or any pharmaceutically acceptable salt or stereoisomer thereof.

In a second embodiment, the invention features a compound having Formula A, wherein:

R^1 and R^2 are each methyl;

n is 0;

R^3 is independently selected from (C_4-C_{20})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from (C_1-C_{16})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from (C_4-C_8) alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from (C_1-C_2)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond or –C(O)O-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

or any pharmaceutically acceptable salt or stereoisomer thereof.

In a third embodiment, the invention features a compound having Formula A, wherein:

R^1 and R^2 are each methyl;

n is 2;

R^3 is independently selected from (C_4-C_{20})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from (C_1-C_{16})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from (C_4-C_8) alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from (C_1-C_2)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond or –C(O)O-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

or any pharmaceutically acceptable salt or stereoisomer thereof.

Specific cationic lipids are:

methyl (9Z)-19-(dimethylamino)octacos-9-enoate (Compound 1);

methyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 2);

methyl (9Z)-19-(dimethylamino)heptacos-9-enoate (Compound 3);

methyl (9Z)-19-(dimethylamino)hexacos-9-enoate (Compound 4);

methyl (9Z)-19-(dimethylamino)pentacos-9-enoate (Compound 5);

methyl (9Z)-21-(dimethylamino)triacont-9-enoate (Compound 6);

methyl (9Z)-21-(dimethylamino)nonacos-9-enoate (Compound 7);

methyl (9Z)-21-(dimethylamino)octacos-9-enoate (Compound 8);

methyl (9Z)-21-(dimethylamino)heptacos-9-enoate (Compound 9);
methyl (11Z)-19-(dimethylamino)octacos-11-enoate (Compound 10);
methyl (7Z)-19-(dimethylamino)octacos-7-enoate (Compound 11);
methyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 12);
methyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 13);
methyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate (Compound 14);
methyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate (Compound 15);
methyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate (Compound 16);
methyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 17);
methyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 18);
methyl 10-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}decanoate (Compound 19);
methyl 6-{2-[11-(dimethylamino)icosyl]cyclopropyl}hexanoate (Compound 20);
ethyl (7Z)-17-(dimethylamino)hexacos-7-enoate (Compound 21);
ethyl (7Z)-17-(dimethylamino)pentacos-7-enoate (Compound 22);
ethyl (7Z)-17-(dimethylamino)tetracos-7-enoate (Compound 23);
ethyl (7Z)-17-(dimethylamino)tricos-7-enoate (Compound 24);
ethyl (9Z)-17-(dimethylamino)hexacos-9-enoate (Compound 25);
ethyl (5Z)-17-(dimethylamino)hexacos-5-enoate (Compound 26);
ethyl (9Z)-19-(dimethylamino)octacos-9-enoate (Compound 27);
ethyl (9Z)-19-(dimethylamino)heptacos-9-enoate (Compound 28);
ethyl (9Z)-19-(dimethylamino)hexacos-9-enoate (Compound 29);
ethyl (9Z)-19-(dimethylamino)pentacos-9-enoate (Compound 30);
ethyl (9Z)-21-(dimethylamino)triacont-9-enoate (Compound 31);
ethyl (9Z)-21-(dimethylamino)nonacos-9-enoate (Compound 32);
ethyl (9Z)-21-(dimethylamino)octacos-9-enoate (Compound 33);
ethyl (9Z)-21-(dimethylamino)heptacos-9-enoate (Compound 34);
ethyl 6-{2-[9-(dimethylamino)octadecyl]cyclopropyl}hexanoate (Compound 35);
ethyl 6-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}hexanoate (Compound 36);
ethyl 6-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}hexanoate (Compound 37);
ethyl 6-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}hexanoate (Compound 38);
ethyl 8-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 39);
ethyl 4-{2-[11-(dimethylamino)icosyl]cyclopropyl}butanoate (Compound 40);
ethyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 41);

ethyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 42);
ethyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 43);
ethyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate (Compound 44);
ethyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate (Compound 45);
ethyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate (Compound 46);
ethyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 47);
ethyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 48);
methyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate (Compound 49);
methyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 50);
methyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate (Compound 51);
methyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate (Compound 52);
methyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate (Compound 53);
methyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate (Compound 54);
methyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate (Compound 55);
methyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate (Compound 56);
methyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate (Compound 57);
methyl (11Z)-19-[(dimethylamino)methyl]octacos-11-enoate (Compound 58);
methyl (7Z)-19-[(dimethylamino)methyl]octacos-7-enoate (Compound 59);
methyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 60);
methyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 61);
methyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate (Compound 62);
methyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate (Compound 63);
methyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate (Compound 64);
methyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 65);
methyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 66);
methyl 10-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)decanoate (Compound 67);
methyl 6-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)hexanoate (Compound 68);
ethyl (7Z)-17-[(dimethylamino)methyl]hexacos-7-enoate (Compound 69);
ethyl (7Z)-17-[(dimethylamino)methyl]pentacos-7-enoate (Compound 70);
ethyl (7Z)-17-[(dimethylamino)methyl]tetracos-7-enoate (Compound 71);
ethyl (7Z)-17-[(dimethylamino)methyl]tricos-7-enoate (Compound 72);
ethyl (9Z)-17-[(dimethylamino)methyl]hexacos-9-enoate (Compound 73);
ethyl (5Z)-17-[(dimethylamino)methyl]hexacos-5-enoate (Compound 74);

ethyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate (Compound 75);
ethyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate (Compound 76);
ethyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate (Compound 77);
ethyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate (Compound 78);
ethyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate (Compound 79);
ethyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate (Compound 80);
ethyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate (Compound 81);
ethyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate (Compound 82);
ethyl 6-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)hexanoate (Compound 83);
ethyl 6-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)hexanoate (Compound 84);
ethyl 6-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)hexanoate (Compound 85);
ethyl 6-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)hexanoate (Compound 86);
ethyl 8-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 87);
ethyl 4-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)butanoate (Compound 88);
ethyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 89);
ethyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 90);
ethyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 91);
ethyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate (Compound 92);
ethyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate (Compound 93);
ethyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate (Compound 94);
ethyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 95);
ethyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 96);
methyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 97);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 98);
methyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 99);
methyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 100);
methyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate (Compound 101);
methyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate (Compound 102);
methyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate (Compound 103);
methyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 104);
methyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 105);
methyl (11Z)-19-[2-(dimethylamino)ethyl]octacos-11-enoate (Compound 106);
methyl (7Z)-19-[2-(dimethylamino)ethyl]octacos-7-enoate (Compound 107);

methyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 108);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 109);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate (Compound 110);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate (Compound 111);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate (Compound 112);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 113);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 114);
methyl 10-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)decanoate (Compound 115);
methyl 6-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)hexanoate (Compound 116);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]hexacos-7-enoate (Compound 117);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]pentacos-7-enoate (Compound 118);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]tetracos-7-enoate (Compound 119);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]tricos-7-enoate (Compound 120);
ethyl (9Z)-17-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 121);
ethyl (5Z)-17-[2-(dimethylamino)ethyl]hexacos-5-enoate (Compound 122);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 123);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 124);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 125);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate (Compound 126);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate (Compound 127);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate (Compound 128);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 129);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 130);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)hexanoate (Compound 131);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)hexanoate (Compound 132);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)hexanoate (Compound 133);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)hexanoate (Compound 134);
ethyl 8-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 135);
ethyl 4-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)butanoate (Compound 136);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 137);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 138);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 139);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate (Compound 140);

ethyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate (Compound 141);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate (Compound 142);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 143);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 144);
(2Z)-non-2-en-1-yl 10-(dimethylamino)nonadecanoate (Compound 145);
(2-hexylcyclopropyl)methyl 10-(dimethylamino)nonadecanoate (Compound 146);
(2Z)-undec-2-en-1-yl 8-(dimethylamino)heptadecanoate (Compound 147);
(2Z)-hept-2-en-1-yl 12-(dimethylamino)henicosanoate (Compound 148);
(2-octylcyclopropyl)methyl 8-(dimethylamino)heptadecanoate (Compound 149);
(2-butylcyclopropyl)methyl 12-(dimethylamino)henicosanoate (Compound 150);
(2Z)-non-2-en-1-yl 10-[(dimethylamino)methyl]nonadecanoate (Compound 151);
(2Z)-undec-2-en-1-yl 8-[(dimethylamino)methyl]heptadecanoate (Compound 152);
(2Z)-hept-2-en-1-yl 12-[(dimethylamino)methyl]henicosanoate (Compound 153);
(2-hexylcyclopropyl)methyl 10-[(dimethylamino)methyl]nonadecanoate (Compound 154);
(2-octylcyclopropyl)methyl 8-[(dimethylamino)methyl]heptadecanoate (Compound 155);
(2-butylcyclopropyl)methyl 12-[(dimethylamino)methyl]henicosanoate (Compound 156);
(2Z)-non-2-en-1-yl 10-[2-(dimethylamino)ethyl]nonadecanoate (Compound 157);
(2Z)-undec-2-en-1-yl 8-[2-(dimethylamino)ethyl]heptadecanoate (Compound 158);
(2Z)-hept-2-en-1-yl 12-[2-(dimethylamino)ethyl]henicosanoate (Compound 159);
(2-hexylcyclopropyl)methyl 10-[2-(dimethylamino)ethyl]nonadecanoate (Compound 160);
(2-octylcyclopropyl)methyl 8-[2-(dimethylamino)ethyl]heptadecanoate (Compound 161);
(2-butylcyclopropyl)methyl 12-[2-(dimethylamino)ethyl]henicosanoate (Compound 162);
methyl (19Z,22Z)-9-(dimethylamino)octacosa-19,22-dienoate (Compound 163);
ethyl (18Z,21Z)-8-(dimethylamino)heptacosa-18,21-dienoate (Compound 164);
methyl 9-(dimethylamino)-16-(2-octylcyclopropyl)hexadecanoate (Compound 165);
ethyl 8-(dimethylamino)-15-(2-octylcyclopropyl)pentadecanoate (Compound 166);
methyl (19Z,22Z)-9-[(dimethylamino)methyl]octacosa-19,22-dienoate (Compound 167);
ethyl (18Z,21Z)-8-[(dimethylamino)methyl]heptacosa-18,21-dienoate (Compound 168);
methyl 9-[(dimethylamino)methyl]-16-(2-octylcyclopropyl)hexadecanoate (Compound 169);
ethyl 8-[(dimethylamino)methyl]-15-(2-octylcyclopropyl)pentadecanoate (Compound 170);
methyl (19Z,22Z)-9-[2-(dimethylamino)ethyl]octacosa-19,22-dienoate (Compound 171);
ethyl (18Z,21Z)-8-[2-(dimethylamino)ethyl]heptacosa-18,21-dienoate (Compound 172);
methyl 9-[2-(dimethylamino)ethyl]-16-(2-octylcyclopropyl)hexadecanoate (Compound 173);

ethyl 8-[2-(dimethylamino)ethyl]-15-(2-octylcyclopropyl)pentadecanoate (Compound 174); dimethyl (9Z)-19-(dimethylamino)heptacos-9-enedioate (Compound 175); and 1-methyl 18-[(2Z)-non-2-en-1-yl] 9-(dimethylamino)octadecanedioate (Compound 176); or any pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment, the cationic lipids disclosed are useful in the preparation of lipid nanoparticles.

In another embodiment, the cationic lipids disclosed are useful components in a lipid nanoparticle for the delivery of oligonucleotides.

In another embodiment, the cationic lipids disclosed are useful components in a lipid nanoparticle for the delivery of siRNA and miRNA.

In another embodiment, the cationic lipids disclosed are useful components in a lipid nanoparticle for the delivery of siRNA.

The cationic lipids of the present invention may have asymmetric centers, chiral axes, and chiral planes (as described in: E.L. Eliel and S.H. Wilen, Stereochemistry of Carbon Compounds, John Wiley & Sons, New York, 1994, pages 1119-1190), and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers and mixtures thereof, including optical isomers, being included in the present invention. In addition, the cationic lipids disclosed herein may exist as tautomers and both tautomeric forms are intended to be encompassed by the scope of the invention, even though only one tautomeric structure is depicted.

It is understood that substituents and substitution patterns on the cationic lipids of the instant invention can be selected by one of ordinary skill in the art to provide cationic lipids that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results.

It is understood that one or more Si atoms can be incorporated into the cationic lipids of the instant invention by one of ordinary skill in the art to provide cationic lipids that are chemically stable and that can be readily synthesized by techniques known in the art from readily available starting materials.

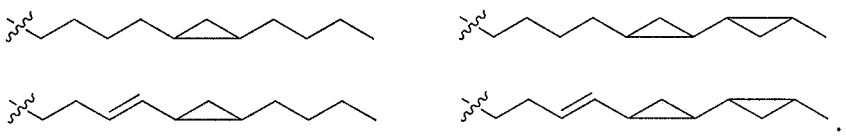
In the compounds of Formula A, the atoms may exhibit their natural isotopic abundances, or one or more of the atoms may be artificially enriched in a particular isotope having the same atomic number, but an atomic mass or mass number different from the atomic

mass or mass number predominantly found in nature. The present invention is meant to include all suitable isotopic variations of the compounds of Formula A. For example, different isotopic forms of hydrogen (H) include protium (^{1}H) and deuterium (^{2}H). Protium is the predominant hydrogen isotope found in nature. Enriching for deuterium may afford certain therapeutic advantages, such as increasing *in vivo* half-life or reducing dosage requirements, or may provide a compound useful as a standard for characterization of biological samples. Isotopically-enriched compounds within Formula A can be prepared without undue experimentation by conventional techniques well known to those skilled in the art or by processes analogous to those described in the Scheme and Examples herein using appropriate isotopically-enriched reagents and/or intermediates.

As used herein, "alkyl" means a straight chain, cyclic or branched saturated aliphatic hydrocarbon having the specified number of carbon atoms.

As used herein, "alkenyl" means a straight chain, cyclic or branched unsaturated aliphatic hydrocarbon having the specified number of carbon atoms including but not limited to diene, triene and tetraene unsaturated aliphatic hydrocarbons.

Examples of a cyclic "alkyl" or "alkenyl include:



As used herein, "heterocyclyl" or "heterocycle" means a 4- to 10-membered aromatic or nonaromatic heterocycle containing from 1 to 4 heteroatoms selected from the group consisting of O, N and S, and includes bicyclic groups. "Heterocyclyl" therefore includes, the following: benzoimidazolyl, benzofuranyl, benzofurazanyl, benzopyrazolyl, benzotriazolyl, benzothiophenyl, benzoxazolyl, carbazolyl, carbolinyl, cinnolinyl, furanyl, imidazolyl, indolinyl, indolyl, indolazinyl, indazolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthpyridinyl, oxadiazolyl, oxazolyl, oxazoline, isoxazoline, oxetanyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridopyridinyl, pyridazinyl, pyridyl, pyrimidyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, tetrahydropyranyl, tetrazolyl, tetrazolopyridyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzoimidazolyl, dihydrobenzofuranyl, dihydrobenzothiophenyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl,

dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidinyl, methylenedioxybenzoyl, tetrahydrofuranyl, and tetrahydrothienyl, and N-oxides thereof all of which are optionally substituted with one to three substituents selected from R".

As used herein, "polyamine" means compounds having two or more amino groups. Examples include putrescine, cadaverine, spermidine, and spermine.

As used herein, "halogen" means Br, Cl, F and I.

In an embodiment of Formula A, R^1 and R^2 are independently selected from H and $(C_1\text{-}C_6)$alkyl, wherein said alkyl is optionally substituted with one to three substituents selected from R', or R^1 and R^2 can be taken together with the nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocyclic heterocycle is optionally substituted with one to three substituents selected from R'.

In an embodiment of Formula A, R^1 and R^2 are independently selected from H, methyl, ethyl and propyl, wherein said methyl, ethyl and propyl are optionally substituted with one to three substituents selected from R', or R^1 and R^2 can be taken together with the nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocyclic heterocycle is optionally substituted with one to three substituents selected from R'.

In an embodiment of Formula A, R^1 and R^2 are independently selected from H, methyl, ethyl and propyl.

In an embodiment of Formula A, R^1 and R^2 are each methyl.

In an embodiment of Formula A, R^3 is independently selected from: $(C_4\text{-}C_{20})$alkyl or alkenyl.

In an embodiment of Formula A, R^3 is independently selected from: $(C_{14}\text{-}C_{18})$ alkenyl.

In an embodiment of Formula A, R^3 is independently selected from: (C_{16}) alkenyl.

In an embodiment of Formula A, R^3 is independently selected from: (C_{14}) alkenyl.

In an embodiment of Formula A, R^3 is independently selected from: (C_{16}) alkenyl.

In an embodiment of Formula A, R^3 is independently selected from: (C_6-C_9)alkyl.

In an embodiment of Formula A, R^3 is independently selected from: (C_7)alkyl.

In an embodiment of Formula A, R^4 is independently selected from: (C_1-C_{16})alkyl or alkenyl.

In an embodiment of Formula A, R^4 is independently selected from: (C_4-C_{10}) alkenyl.

In an embodiment of Formula A, R^4 is independently selected from: (C_9) alkenyl.

In an embodiment of Formula A, R^4 is independently selected from: (C_1-C_4)alkyl.

In an embodiment of Formula A, R^4 is independently selected from: (C_1-C_2)alkyl.

In an embodiment of Formula A, R^4 is (C_2)alkyl.

In an embodiment of Formula A, R^4 is (C_1)alkyl.

In an embodiment of Formula A, R^3 is (C_7)alkyl and R^4 is (C_9)alkenyl.

In an embodiment of Formula A, R^3 is (C_{14})alkenyl and R^4 is (C_2)alkyl.

In an embodiment of Formula A, R^3 is (C_{16})alkenyl and R^4 is (C_1)alkyl.

In an embodiment of Formula A, R^5 is independently selected from (C_4-C_8) alkyl or alkenyl.

In an embodiment of Formula A, R^5 is independently selected from (C_4-C_8) alkyl.

In an embodiment of Formula A, R^5 is independently selected from (C_6-C_8) alkyl.

In an embodiment of Formula A, R^5 is (C8)alkyl.

In an embodiment of Formula A, R^6 is independently selected from (C_1-C_2) alkyl.

In an embodiment of Formula A, R^6 is (C2)alkyl.

In an embodiment of Formula A, R^6 is (C1)alkyl.

In an embodiment of Formula A, R^5 is (C_8)alkyl and R^4 is (C_2)alkyl.

In an embodiment of Formula A, R^5 is (C_8)alkyl and R^4 is (C_1)alkyl.

In an embodiment of Formula A, R^5 is (C_7)alkyl and R^4 is (C_2)alkyl.

In an embodiment of Formula A, R^5 is (C_7)alkyl and R^4 is (C_1)alkyl.

In an embodiment of Formula A, Q^1 and Q^2 are each, independently a bond, -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -S-S-, -C(R")=N-, -N=C(R")-, -C(R")=N-O-, -O-N=C(R")-, -C(O)(NR")-, -N(R")C(O)-, C(S)(NR")-, -N(R")C(O)-, -N(R")C(O)N(R")-, -OC(O)O-, $OSi(R")_2O$-, -C(O)($CR"_2$)C(O)O-, or –OC(O)($CR"_2$)C(O)-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond.

In an embodiment of Formula A, Q^1 and Q^2 are each, independently a bond or –C(O)O-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond.

In an embodiment of Formula A, R' is R".

In an embodiment of Formula A, R" is independently selected from H, methyl, ethyl and propyl, wherein said methyl, ethyl and propyl are optionally substituted with one or more halogen and OH.

In an embodiment of Formula A, R" is independently selected from H, methyl, ethyl and propyl.

In an embodiment of Formula A, n is 0, 1, 2 or 3.

In an embodiment of Formula A, n is 0, 1 or 2.

In an embodiment of Formula A, n is 0.

In an embodiment of Formula A, n is 1.

In an embodiment of Formula A, n is 2.

In an embodiment of Formula A, "heterocyclyl" is pyrolidine, piperidine, morpholine, imidazole or piperazine.

In an embodiment of Formula A, "monocyclic heterocyclyl" is pyrolidine, piperidine, morpholine, imidazole or piperazine.

In an embodiment of Formula A, "polyamine" is putrescine, cadaverine, spermidine or spermine.

In an embodiment, "alkyl" is a straight chain saturated aliphatic hydrocarbon having the specified number of carbon atoms.

In an embodiment, "alkenyl" is a straight chain unsaturated aliphatic hydrocarbon having the specified number of carbon atoms.

Included in the instant invention is the free form of cationic lipids of Formula A, as well as the pharmaceutically acceptable salts and stereoisomers thereof. Some of the isolated specific cationic lipids exemplified herein are the protonated salts of amine cationic lipids. The term "free form" refers to the amine cationic lipids in non-salt form. The encompassed pharmaceutically acceptable salts not only include the isolated salts exemplified for the specific cationic lipids described herein, but also all the typical pharmaceutically acceptable salts of the free form of cationic lipids of Formula A. The free form of the specific salt cationic lipids described may be isolated using techniques known in the art. For example, 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 free forms may differ from their respective salt forms somewhat in certain physical properties, such as solubility in polar solvents, but the acid and base salts are otherwise pharmaceutically equivalent to their respective free forms for purposes of the invention.

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 the conventional 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, conventional 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, trifluoroacetic (TFA) and the like.

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, zinc and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. 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, tromethamine and the like.

The preparation of the pharmaceutically acceptable salts described above and other typical pharmaceutically acceptable salts is more fully described by Berg *et al.*, "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977:66:1-19.

It will also be noted that the cationic lipids of the present invention are potentially 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.

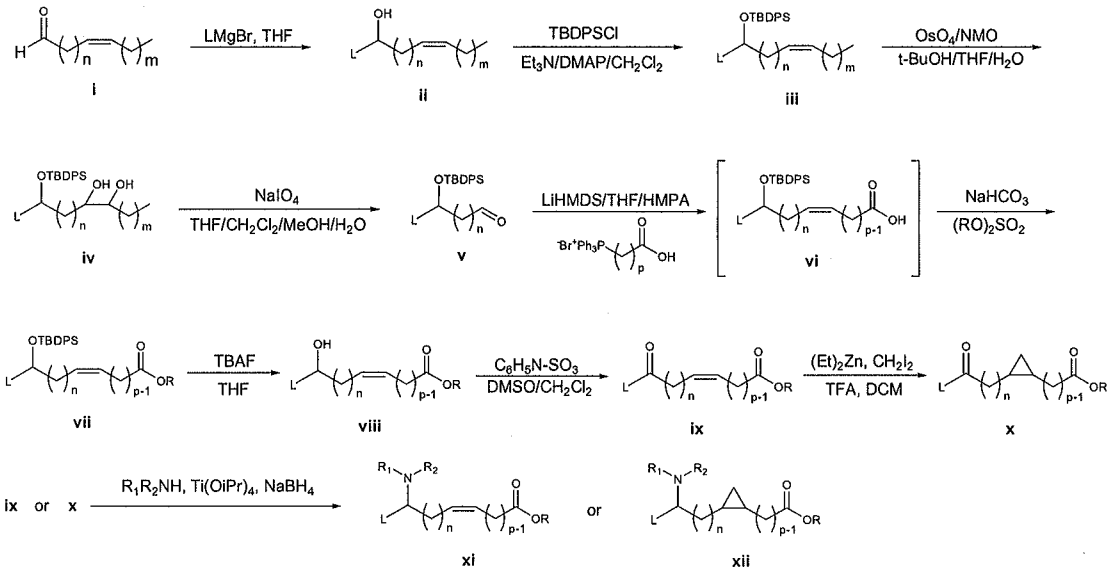
EXAMPLES

Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and not limitative of the reasonable scope thereof. The reagents utilized in synthesizing the cationic lipids are either commercially available or readily prepared by one of ordinary skill in the art.

Synthesis of the novel cationic lipids is a linear process starting from lipid acid (**i**). Addition of a lipid based Grignard reagent can generate secondary alcohol (**ii**). This alcohol is protected as its silyl ether (**iii**) and the olefin is dihydroxylated with osmium tetroxide to give diol (**iv**). The diol is oxidatively cleaved with sodium periodate to provide aldehyde (**v**). The aldehyde is converted to the carboxylic acid containing olefin (**vi**) by a Wittig olenfination. The acid is converted to the ester (**vii**) in situ, followed by silyl ether deprotection to give alcohol (**viii**). The alcohol is oxidized to the ketone (**ix**) which is further converted to the cyclopropane

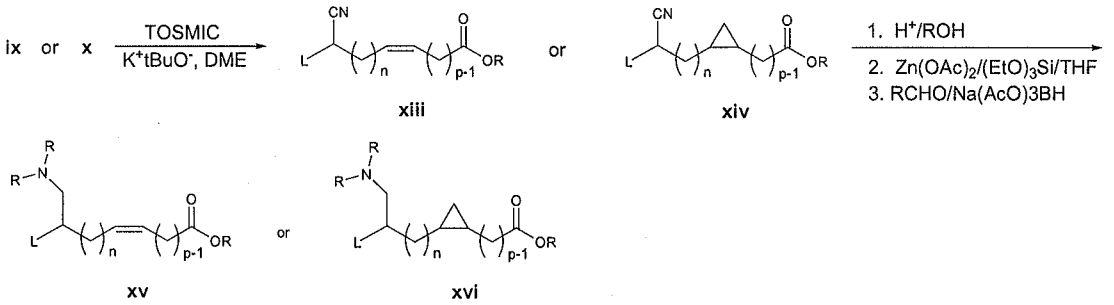
containing material (**x**). Either ketone (**ix** or **x**) is reductively aminated to give final cationic lipids (**xi** or **xii**, respectively).

GENERAL SCHEME 1



Synthesis of the single carbon homologated cationic lipids **xv** and **xvi** is a linear process starting from lipid ketones (**ix** or **x**). Conversion of the ketone to the nitrile (**xiii** or **xiv**) is accomplished via treatment with TOSMIC and potassium *tert*-butoxide. Hydrolysis of the nitrile is achieved under acidic conditions to give the primary amide. The amide is selectively reduced to the primary amine and this is reductively aminated to give amines (**xv** or **xvi**)

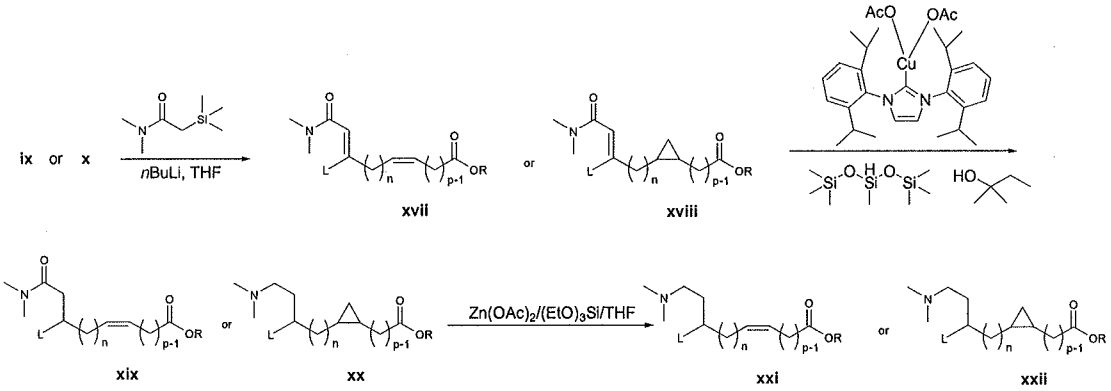
GENERAL SCHEME 2



Synthesis of two carbon homologated cationic lipids **xxi** or **xxii** is a linear process starting from lipid ketone (**ix** or **x**). Conversion of the ketone to the α,β-unsaturated amide **xvii**

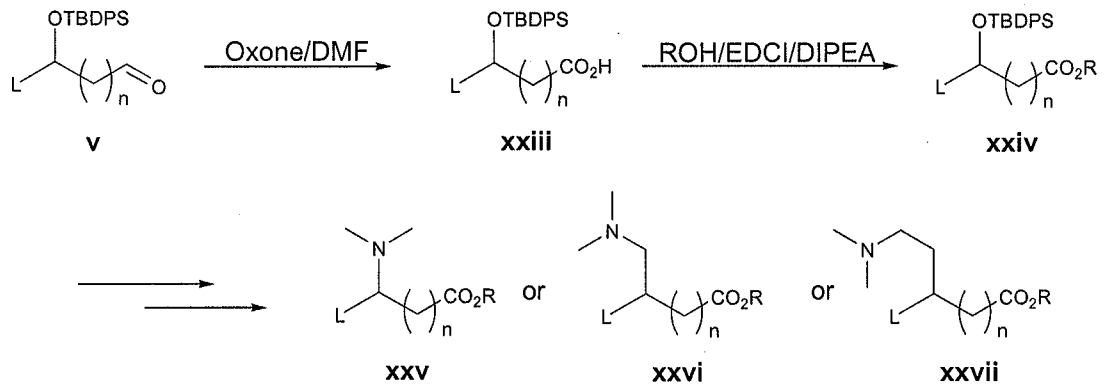
or **xviii** is accomplished under Peterson conditions. Conjugate reduction of the α,β-unsaturation is performed to give amide **xix** or **xx**. Chemoselective reduction of the amide could provide final cationic lipids **xxi** or **xxii**.

GENERAL SCHEME 3



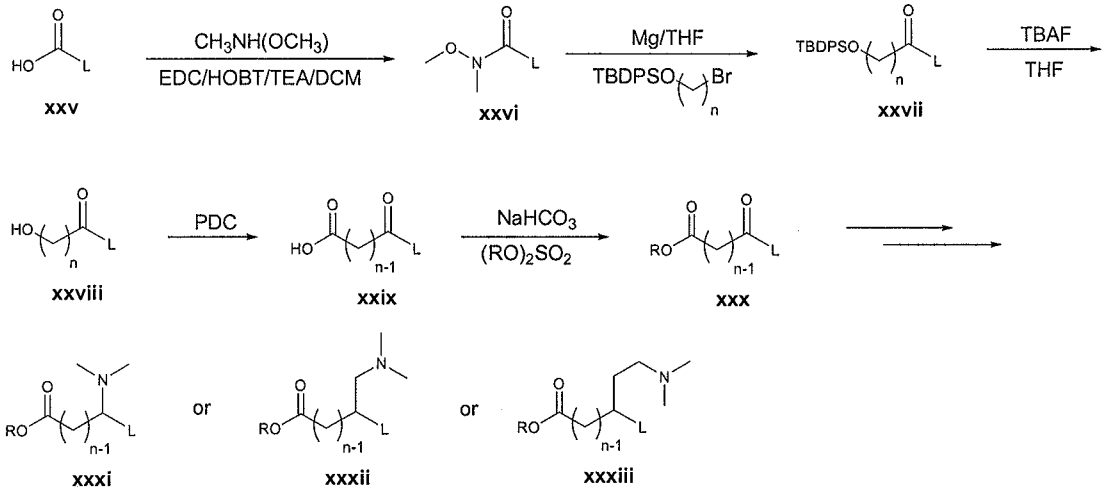
Synthesis of ester containing lipids (**xxv**, **xxvi**, and **xxvii**) is achieved by oxidation of aldehyde **v** to carboxylic acid **xxiii**, followed by ester formation. Conversion to **xxv**, **xxvi** or **xxvii** is completed in a manner analogous to that described in General Schemes 1-3.

GENERAL SCHEME 4



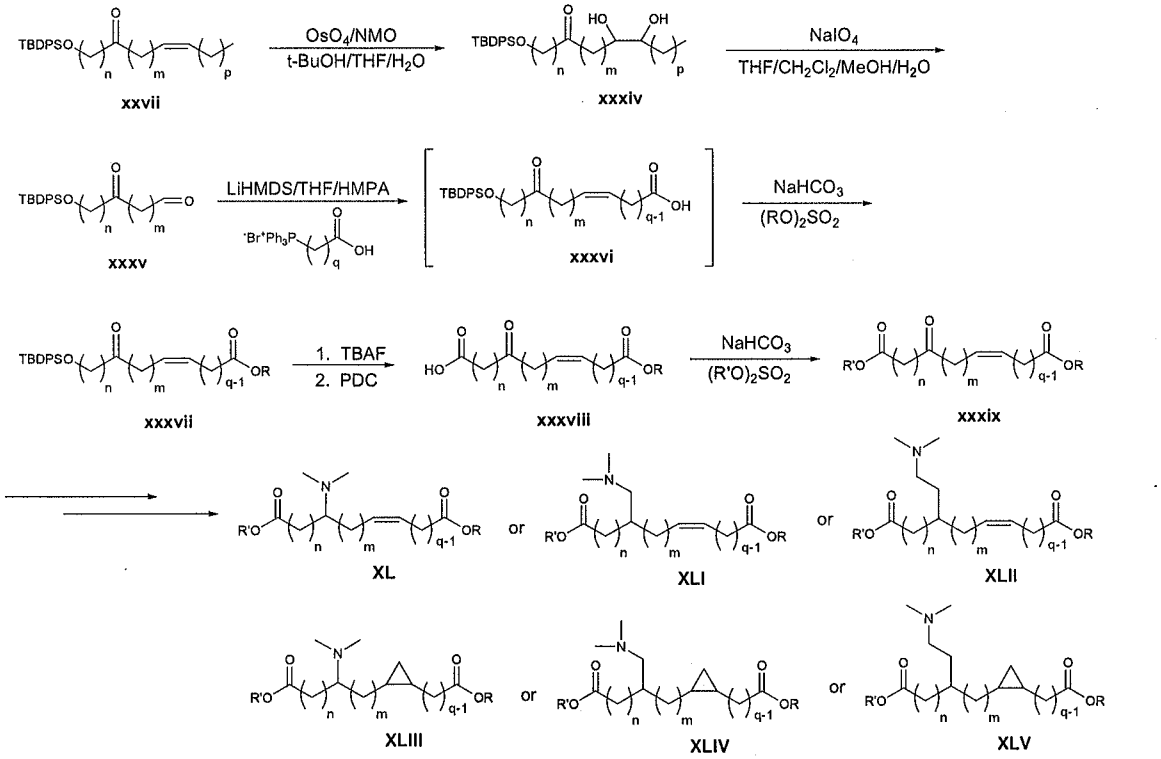
Synthesis of ester containing lipids **xxxi**, **xxxii** and **xxxiii** is a linear sequence beginning with carboxylic acid **xxv**. The acid is converted to the Weinreb amide **xxvi** followed by Grignard addition to give ketone **xxvii**. The alcohol is deprotected, oxidized and esterified to give ester **xxx**. Ketone **xxx** is converted to final amines **xxxi**, **xxxii** or **xxxiii** in a manner analogous to that outlined above in General Schemes 1-3.

GENERAL SCHEME 5



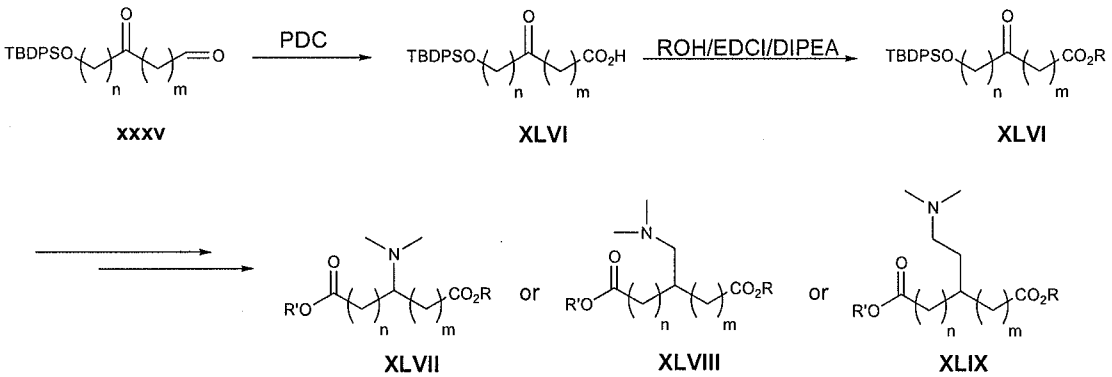
Synthesis of diester amines is accomplished as outlined in General Scheme 6. Alkene **xxvi** is dihydroxylated and oxidatively cleaved to give aldehyde **xxxv**. The aldehyde is converted to the carboxylic acid containing alkene via a Wittig olefination. The resulting acid **xxxvi** is converted to its corresponding ester in situ. The silyl ether is deprotected and the alcohol oxidized to give carboxylic acid **xxxviii**. This is esterified to give ketone intermediate **xxxix**. This ketone is carried on to final amines **XL-XLV** as outlined in General Schemes 1-3 above.

GENERAL SCHEME 6

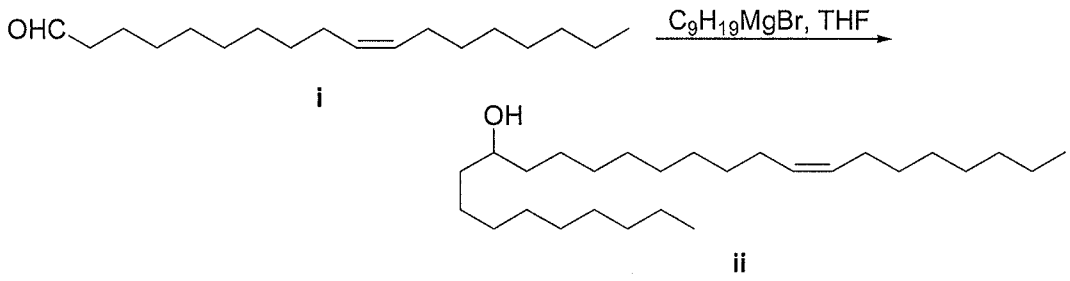


Synthesis of bis-ester compounds (**XLVII-XLIX**) is achieved by oxidation of aldehyde (**xxxv**) followed by esterification to give ester **XLVI**. Conversion to the final amines is achieved in a manner analogous to that described above in General Schemes 1-6.

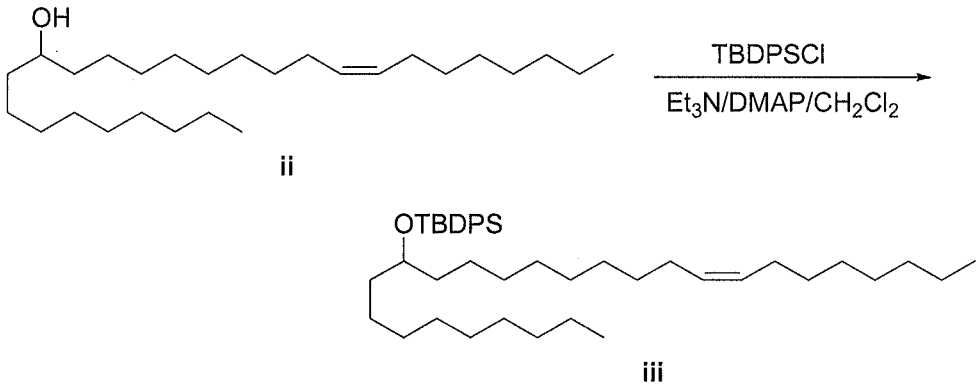
GENERAL SCHEME 7



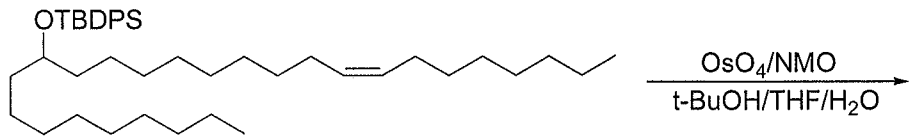
Methyl (9Z)-19-(dimethylamino)octacos-9-enoate (Compound 1)



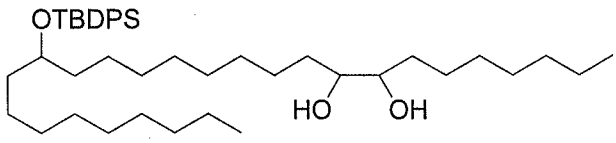
Oleyl aldehyde in THF is cooled to 0 °C and treated with nonylmagnesium bromide. The reaction is warmed to room temperature and quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude alcohol **ii**. The crude product is purified by flash column chromatography.



Alcohol **ii** is taken up in dichloromethane and treated with triethyl amine and DMAP. To this solution is added TBDPSCl in a single portion at ambient temperature. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude silyl ether **iii**. The crude product is purified by flash column chromatography.

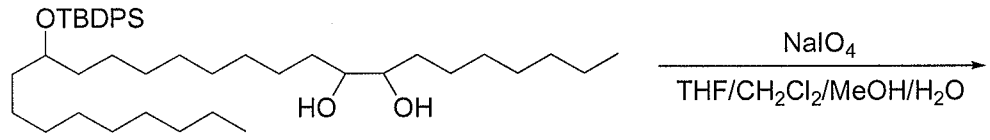


iii

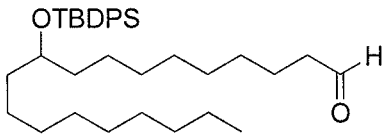


iv

Silyl ether **iii** is taken up in a mixture of tert-butanol, THF and water and treated with osmium tetroxide and NMO. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude diol **iv**. The crude product is purified by flash column chromatography.

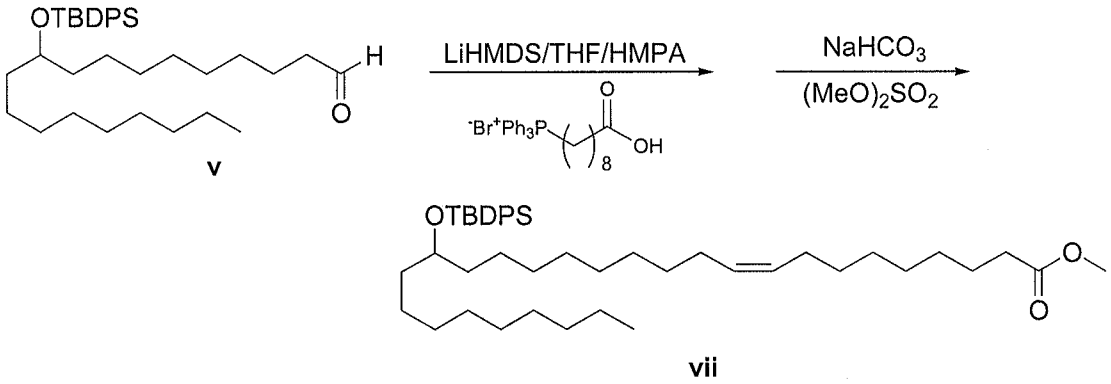


iv



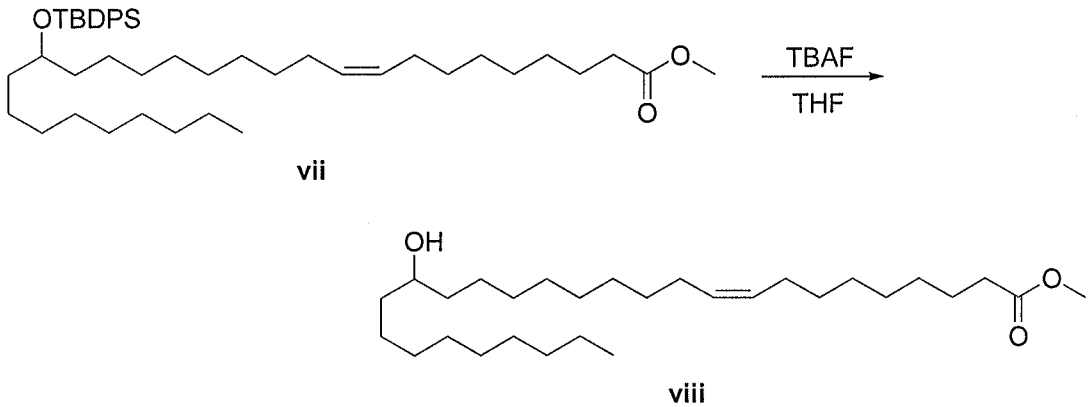
v

Diol **iv** is taken up in a mixture of THF, dichloromethane, methanol and water and treated with sodium periodate. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude aldehyde **v**. The crude product is purified by flash column chromatography.



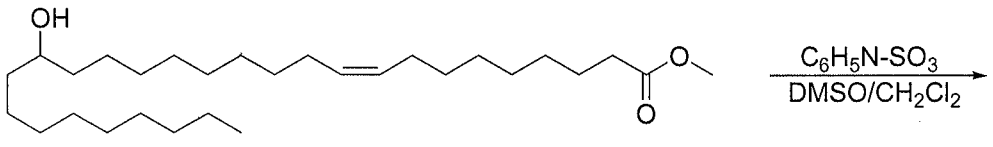
vii

Ylide precursor triphenylphosphinium bromide is taken up in THF and treated with HMPA and lithium hexamethyldisilazide to generate the ylide. To this solution is added aldehyde **v**. Upon reaction completion, the solution is treated with sodium bicarbonate and dimethylsulfate. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude ester **vii**. The crude product is purified by flash column chromatography.

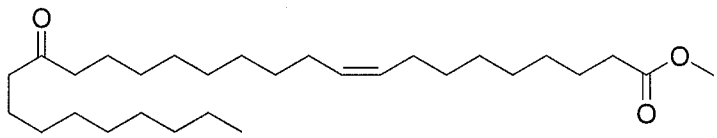


viii

Ester **vii** is taken up in THF and treated with TBAF. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude alcohol **viii**. The crude product is purified by flash column chromatography.

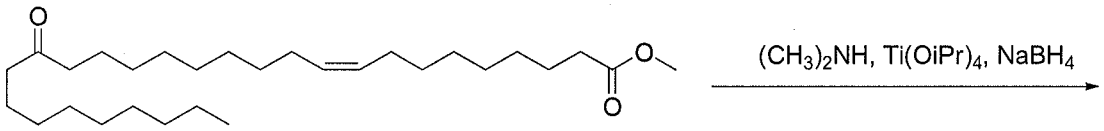


viii



ix

Alcohol **viii** is taken up in a mixture of DMSO and dichloromethane and treated with SO3-pyridine at ambient temperature. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude ketone **ix**. The crude product is purified by flash column chromatography.



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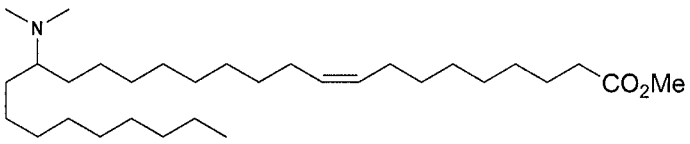
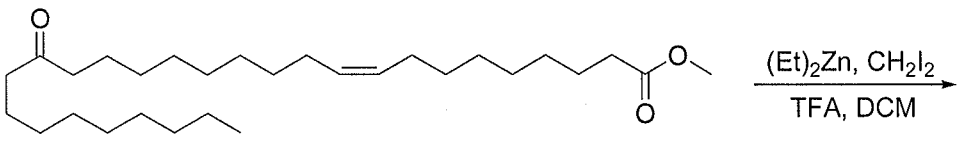


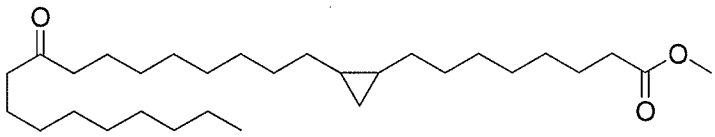
1

A solution of ketone **ix** in THF is treated with dimethylamine, titanium isopropoxide and sodium borohydride. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude amine **1**. The crude product is purified by flash column chromatography.

Methyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 2)

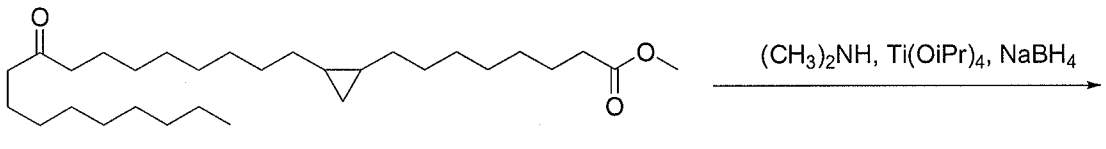


ix

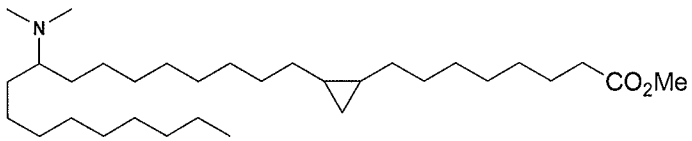


x

A solution of diethylzinc in dichloromethane is cooled to -1 °C and treated dropwise with TFA. After 30 minutes, diiodomethane is added and the resulting solution aged for 30 minutes in an ice bath. To this solution is added ketone **ix** and the resulting solution is warmed slowly to ambient temperature. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude cyclopropane **x**. The crude product is purified by flash column chromatography.



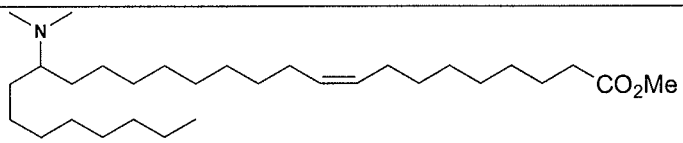
x

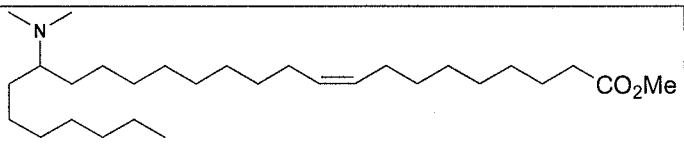
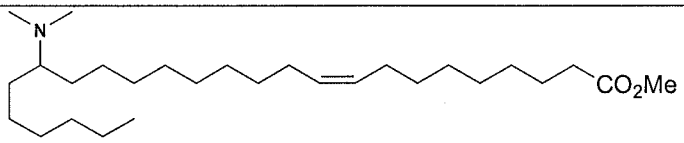
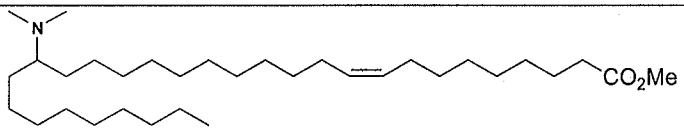
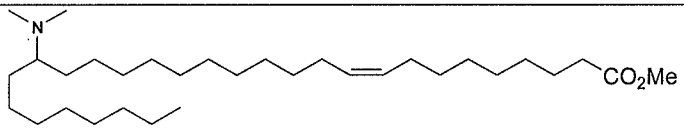
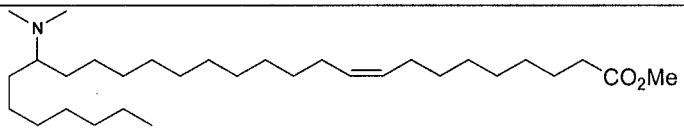
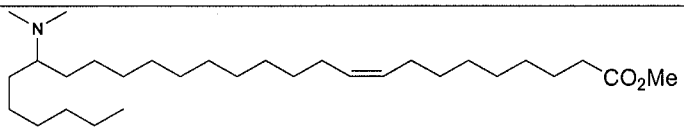
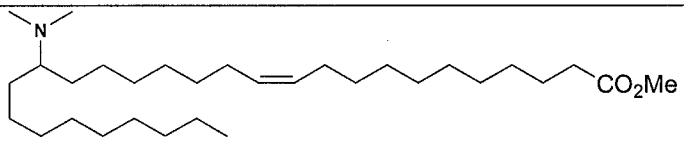
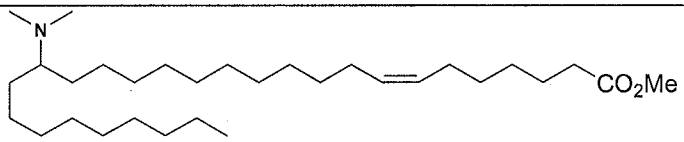
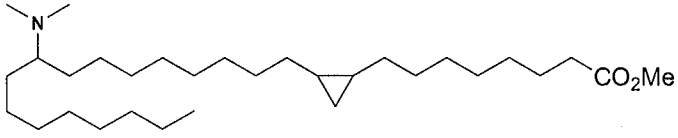
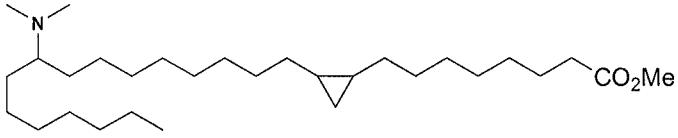


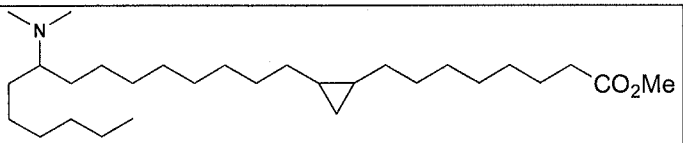
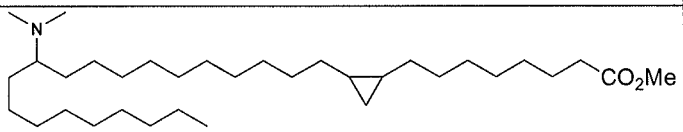
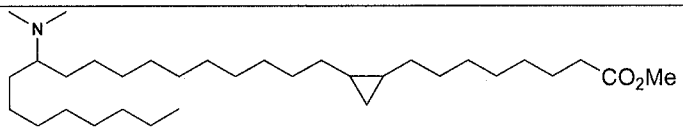
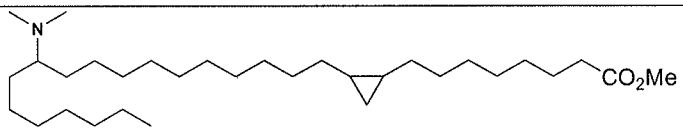
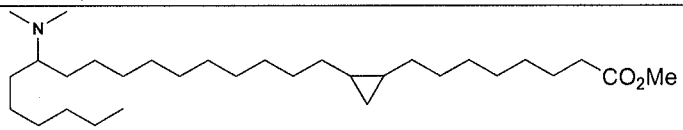
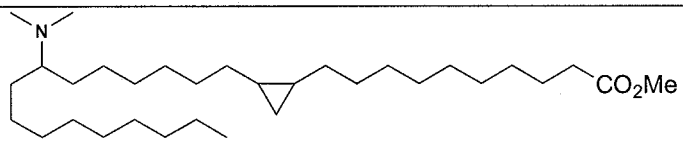
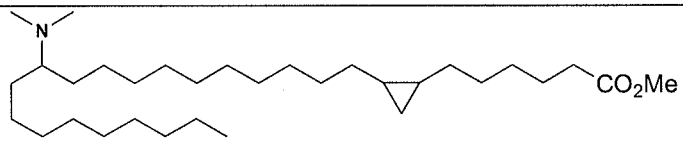
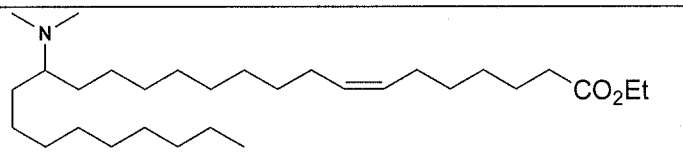
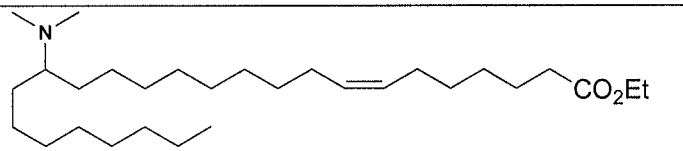
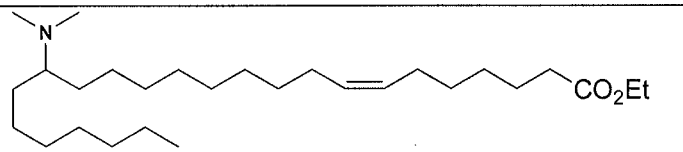
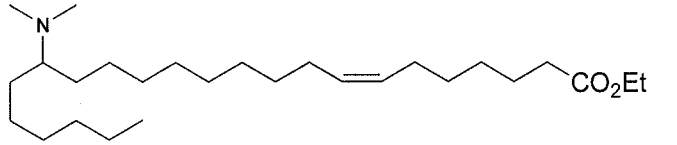
2

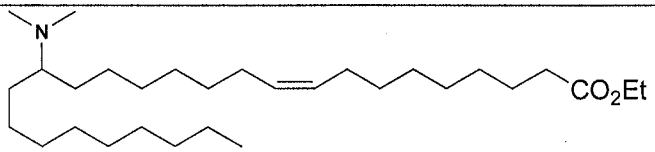
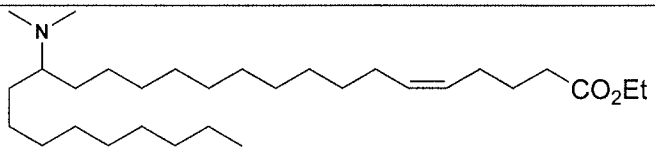
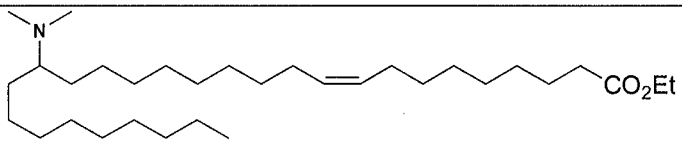
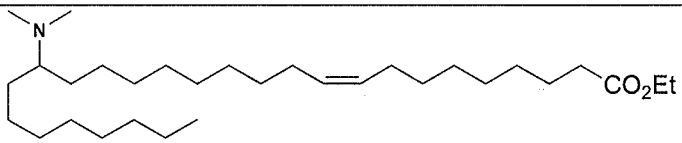
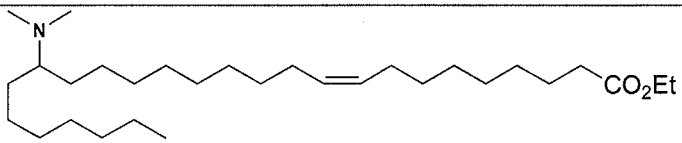
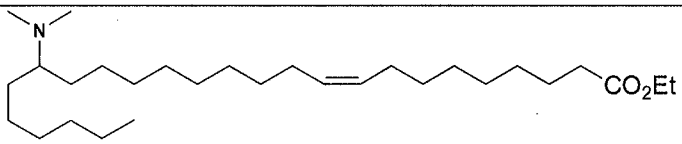
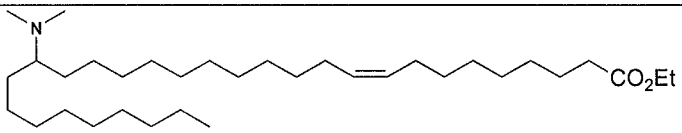
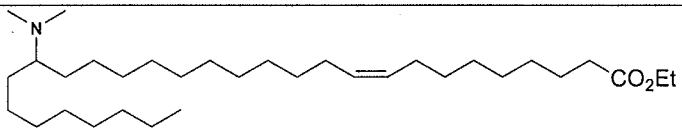
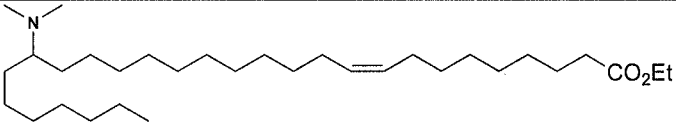
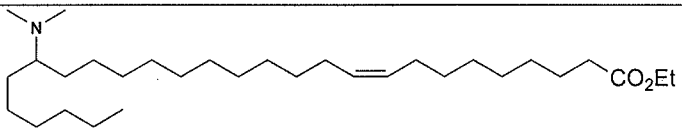
Ketone **x** is carried on to amine **2** as described for compound **1** above.

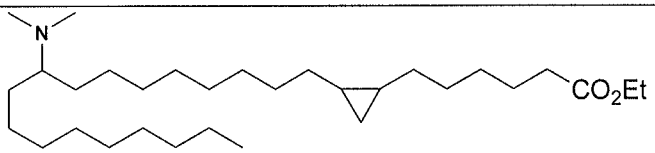
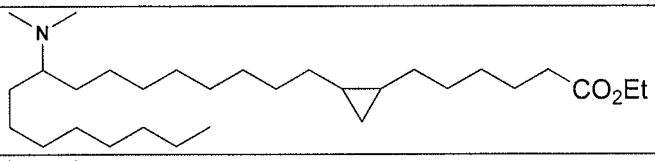
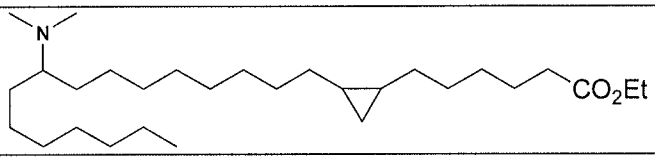
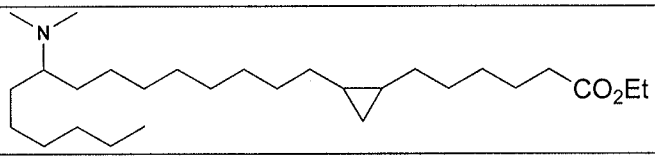
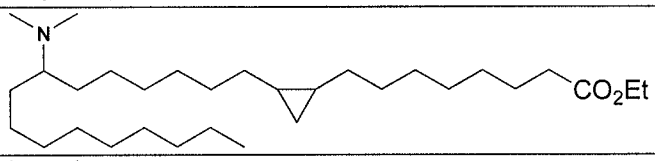
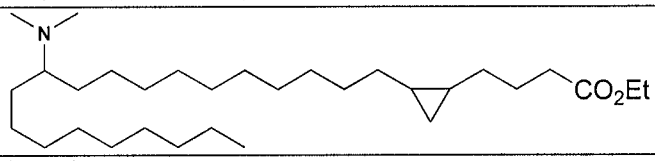
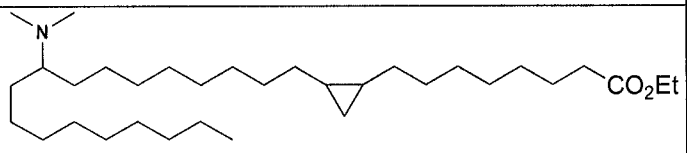
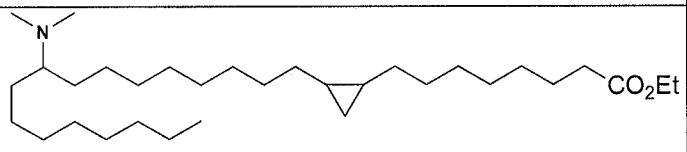
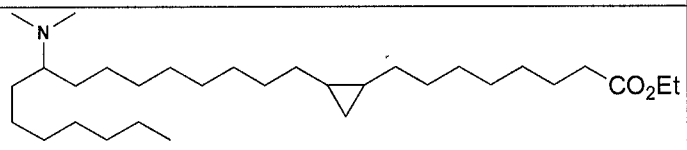
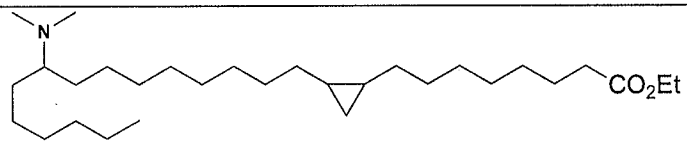
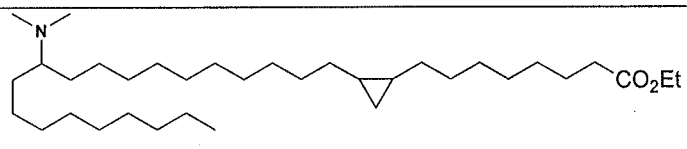
Compounds 3-48 are novel cationic lipids and are prepared according to the General Scheme 1 above.

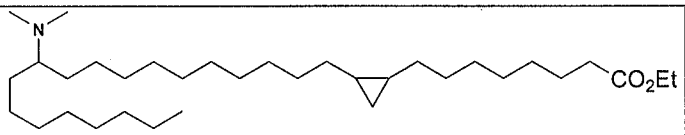
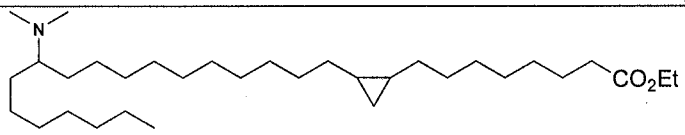
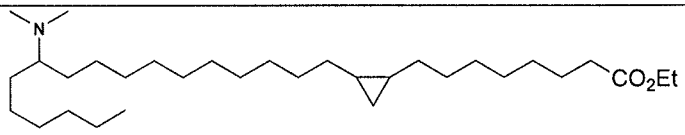
Compound	Structure	Name
3		methyl (9Z)-19-(dimethylamino)heptacos-9-enoate

4		methyl (9Z)-19-(dimethylamino)hexacos-9-enoate
5		methyl (9Z)-19-(dimethylamino)pentacos-9-enoate
6		methyl (9Z)-21-(dimethylamino)triacont-9-enoate
7		methyl (9Z)-21-(dimethylamino)nonacos-9-enoate
8		methyl (9Z)-21-(dimethylamino)octacos-9-enoate
9		methyl (9Z)-21-(dimethylamino)heptacos-9-enoate
10		methyl (11Z)-19-(dimethylamino)octacos-11-enoate
11		methyl (7Z)-19-(dimethylamino)octacos-7-enoate
12		methyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate
13		methyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate

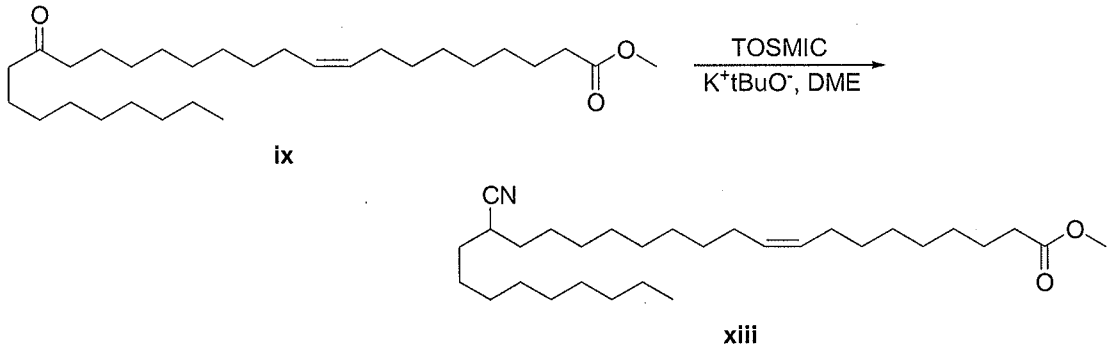
14		methyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate
15		methyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate
16		methyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate
17		methyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate
18		methyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate
19		methyl 10-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}decanoate
20		methyl 6-{2-[11-(dimethylamino)icosyl]cyclopropyl}hexanoate
21		ethyl (7Z)-17-(dimethylamino)hexacos-7-enoate
22		ethyl (7Z)-17-(dimethylamino)pentacos-7-enoate
23		ethyl (7Z)-17-(dimethylamino)tetracos-7-enoate
24		ethyl (7Z)-17-(dimethylamino)tricos-7-enoate

25		ethyl (9Z)-17-(dimethylamino)hexacos-9-enoate
26		ethyl (5Z)-17-(dimethylamino)hexacos-5-enoate
27		ethyl (9Z)-19-(dimethylamino)octacos-9-enoate
28		ethyl (9Z)-19-(dimethylamino)heptacos-9-enoate
29		ethyl (9Z)-19-(dimethylamino)hexacos-9-enoate
30		ethyl (9Z)-19-(dimethylamino)pentacos-9-enoate
31		ethyl (9Z)-21-(dimethylamino)triacont-9-enoate
32		ethyl (9Z)-21-(dimethylamino)nonacos-9-enoate
33		ethyl (9Z)-21-(dimethylamino)octacos-9-enoate
34		ethyl (9Z)-21-(dimethylamino)heptacos-9-enoate

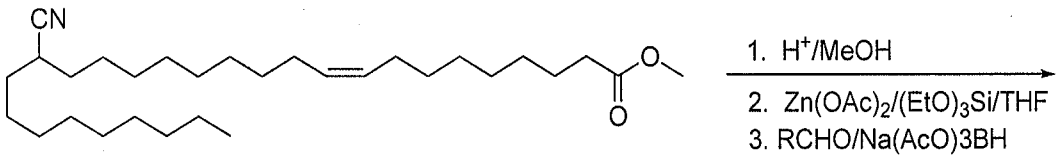
35		ethyl 6-{2-[9-(dimethylamino)octadecyl]cyclopropyl}hexanoate
36		ethyl 6-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}hexanoate
37		ethyl 6-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}hexanoate
38		ethyl 6-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}hexanoate
39		ethyl 8-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}octanoate
40		ethyl 4-{2-[11-(dimethylamino)icosyl]cyclopropyl}butanoate
41		ethyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate
42		ethyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate
43		ethyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate
44		ethyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate
45		ethyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate

46		ethyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate
47		ethyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate
48		ethyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate

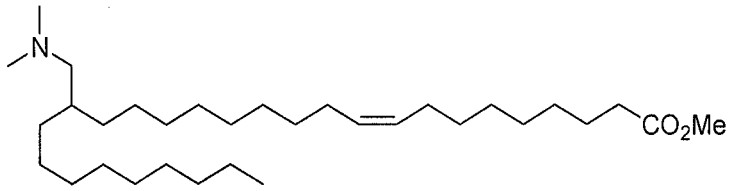
Methyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate (Compound 49)



A solution of ketone **ix** is treated with TOSMIC in dimethoxyethane at 0 °C followed by treatment with potassium tert-butoxide. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude nitrile **xiii**. The crude product is purified by flash column chromatography.



xiii

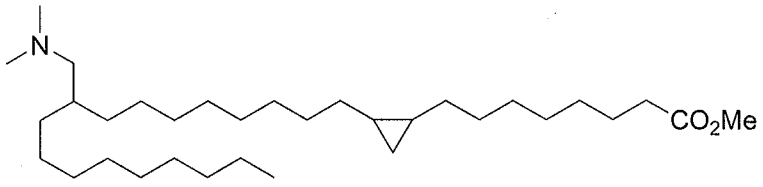


49

A solution of nitrile **xiii** in methanol is treated with concentrated hydrochloric acid and warmed to reflux. The reaction is evaporated *in vacuo* upon completion (conversion to primary amide). The crude amide is selectively reduced by dilution in THF and treatment with zinc acetate and triethoxysilane. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude primary amine. This material is purified by flash chromatography.

The primary amine is taken up in THF and treated with formaldehyde and sodium triacetoxyborohydride. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude amine **49**. The crude product is purified by flash column chromatography.

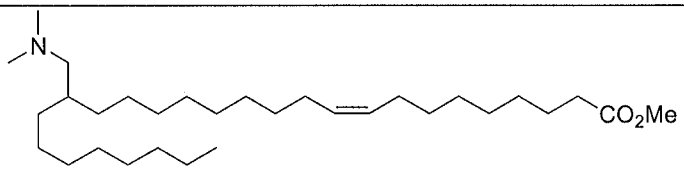
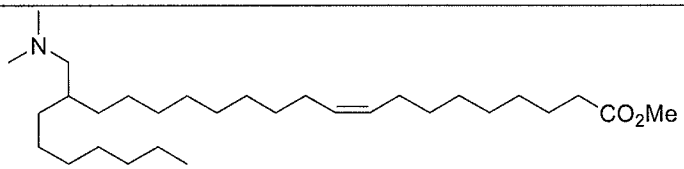
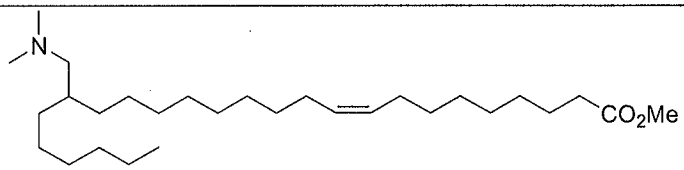
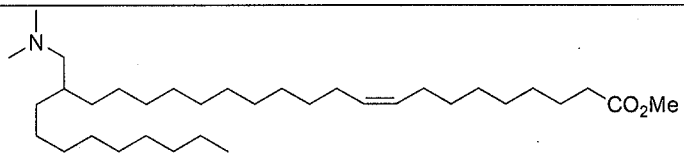
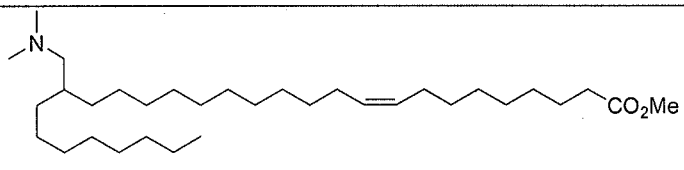
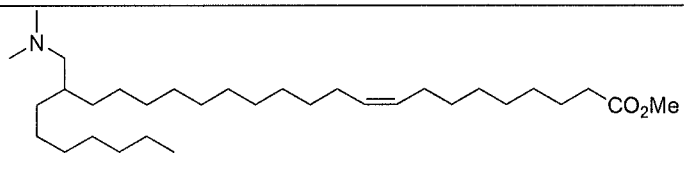
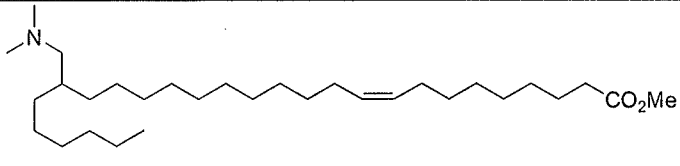
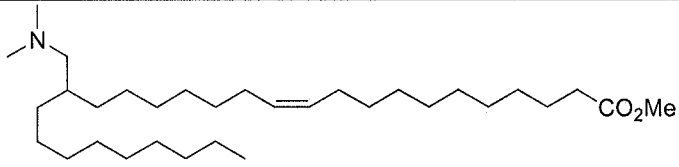
Methyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 50)

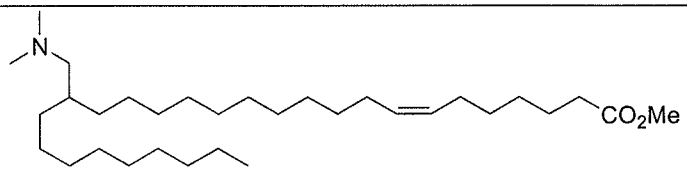
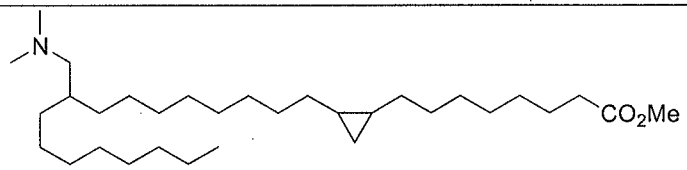
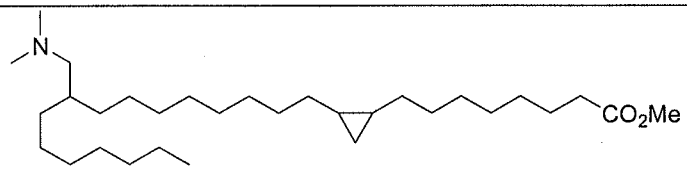
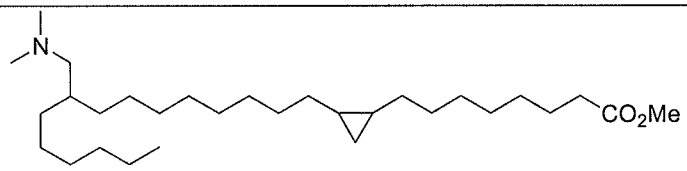
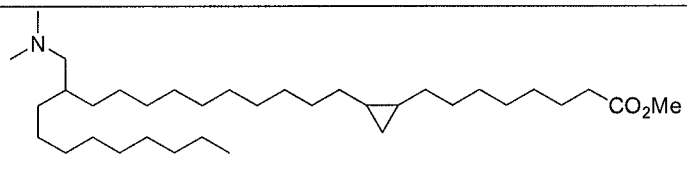
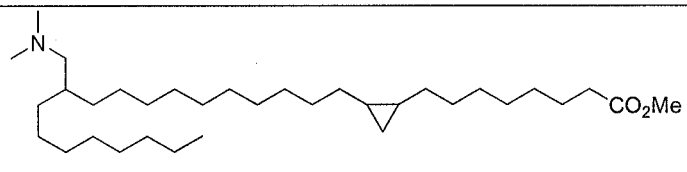
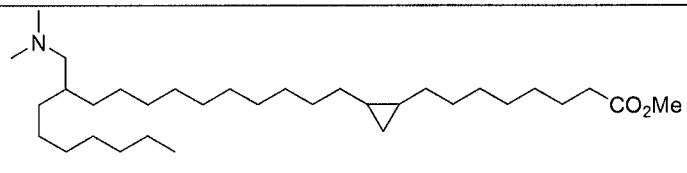
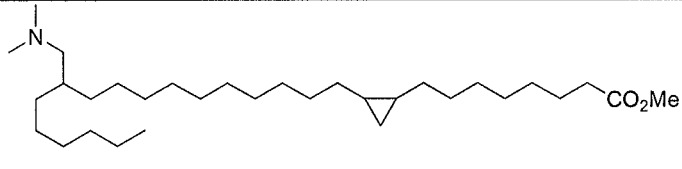


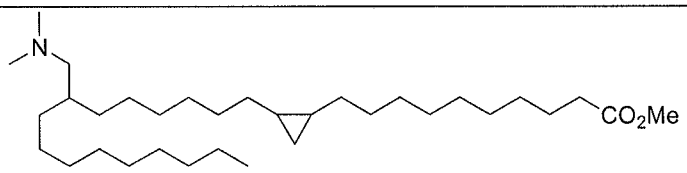
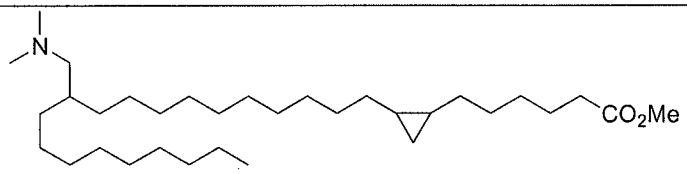
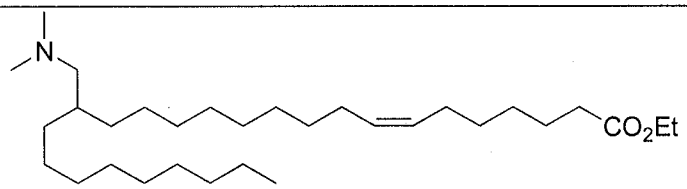
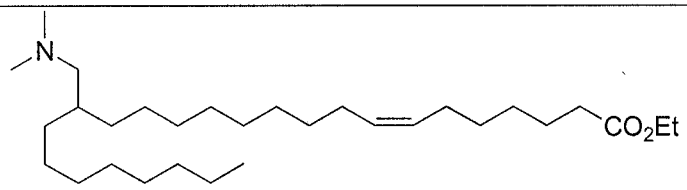
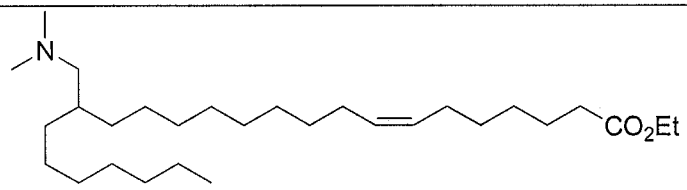
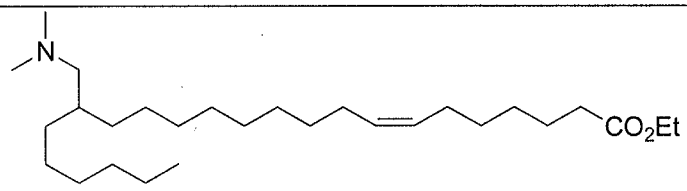
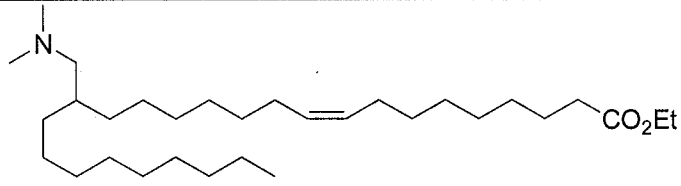
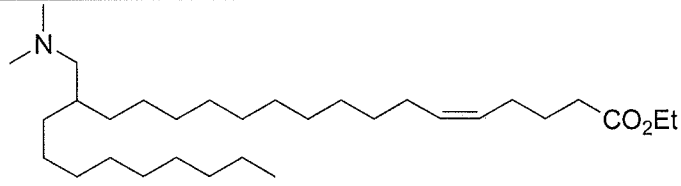
50

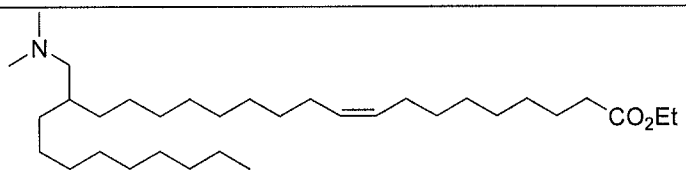
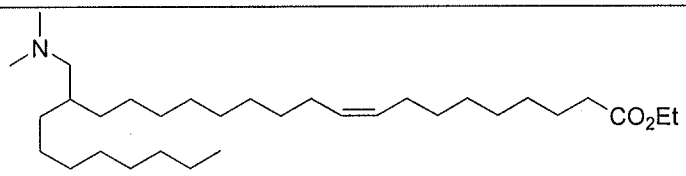
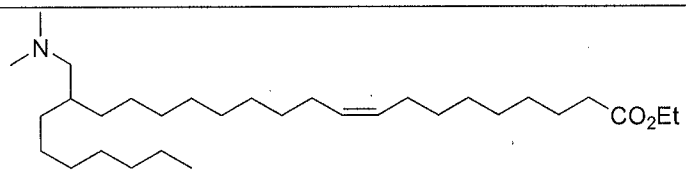
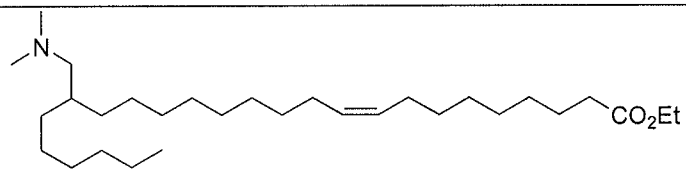
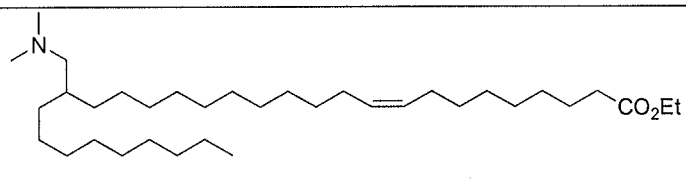
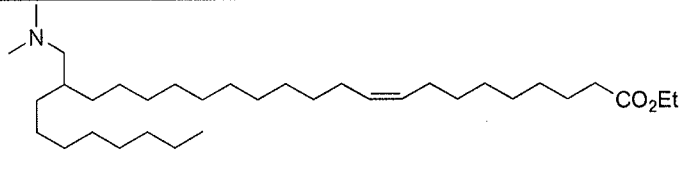
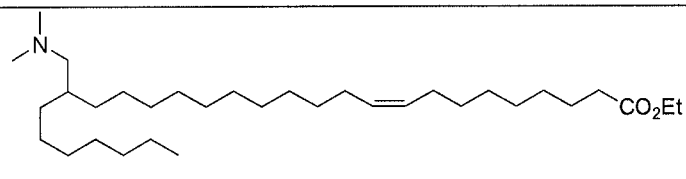
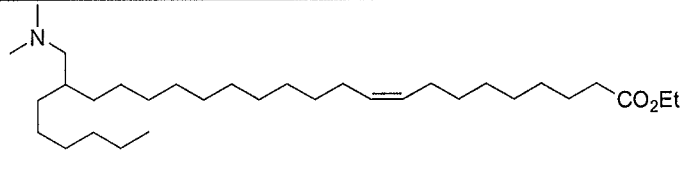
Amine **50** is prepared from ketone **x** in a manner analogous to that outlined above for compound **49**.

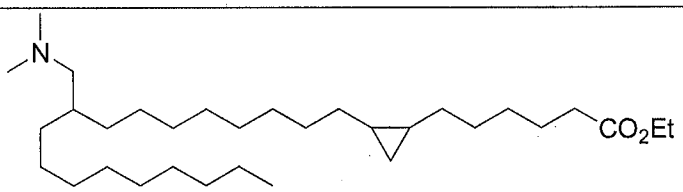
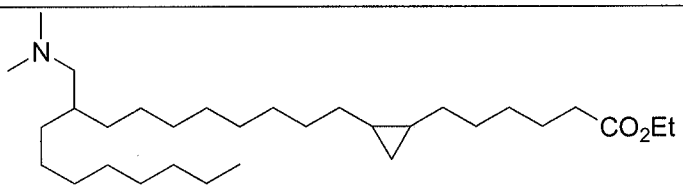
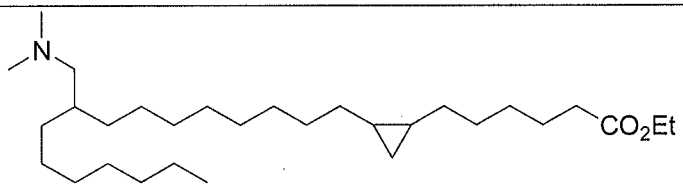
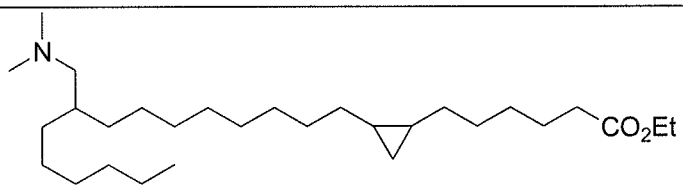
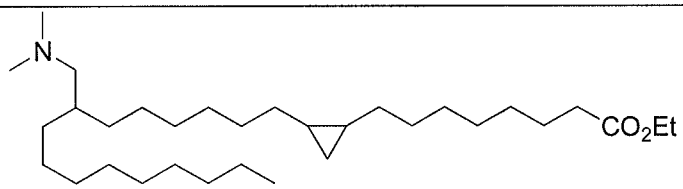
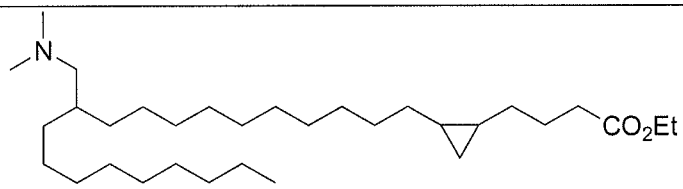
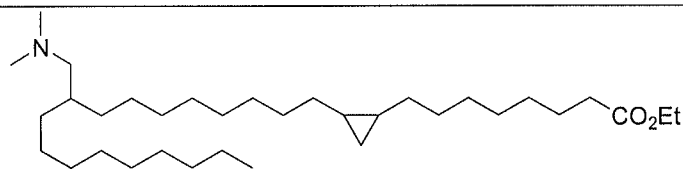
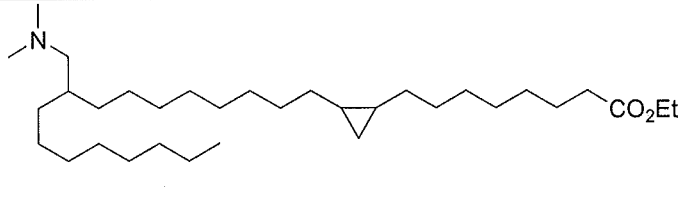
Compounds 51-96 are novel cationic lipids and are prepared according to the General Scheme 2 above.

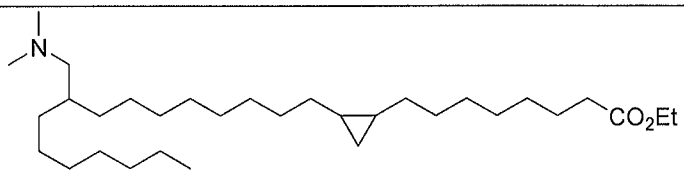
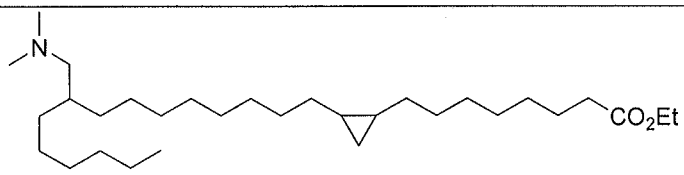
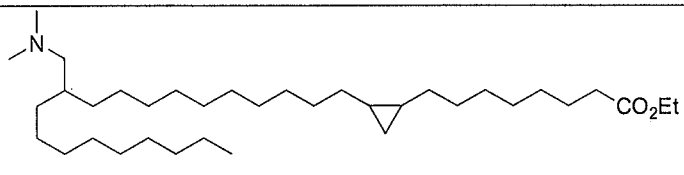
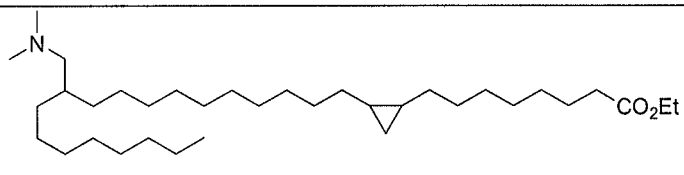
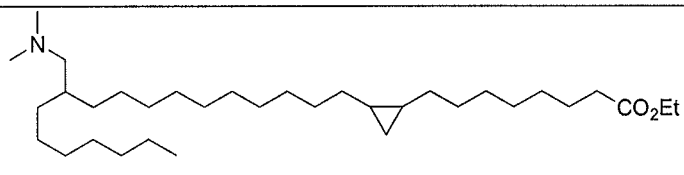
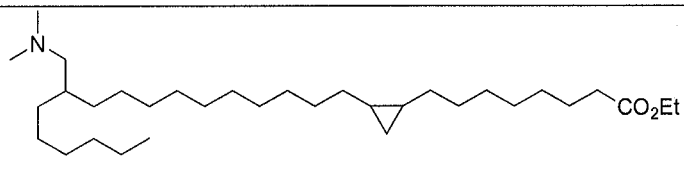
Compound	Structure	Name
51		methyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate
52		methyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate
53		methyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate
54		methyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate
55		methyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate
56		methyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate
57		methyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate
58		methyl (11Z)-19-[(dimethylamino)methyl]octacos-11-enoate

59		methyl (7Z)-19-[(dimethylamino)methyl]octacos-7-enoate
60		methyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate
61		methyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate
62		methyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate
63		methyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate
64		methyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate
65		methyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate
66		methyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate

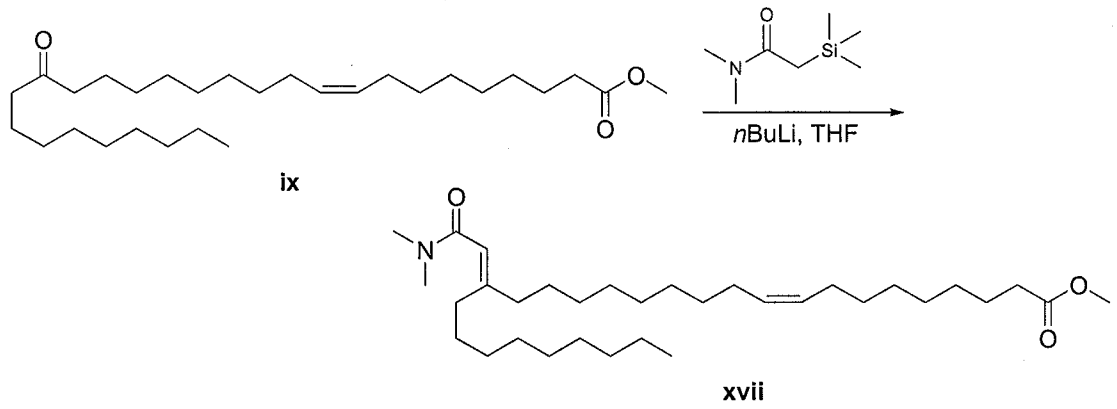
67		methyl 10-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)decanoate
68		methyl 6-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)hexanoate
69		ethyl (7Z)-17-[(dimethylamino)methyl]hexacos-7-enoate
70		ethyl (7Z)-17-[(dimethylamino)methyl]pentacos-7-enoate
71		ethyl (7Z)-17-[(dimethylamino)methyl]tetracos-7-enoate
72		ethyl (7Z)-17-[(dimethylamino)methyl]tricos-7-enoate
73		ethyl (9Z)-17-[(dimethylamino)methyl]hexacos-9-enoate
74		ethyl (5Z)-17-[(dimethylamino)methyl]hexacos-5-enoate

75		ethyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate
76		ethyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate
77		ethyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate
78		ethyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate
79		ethyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate
80		ethyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate
81		ethyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate
82		ethyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate

83		ethyl 6-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)hexanoate
84		ethyl 6-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)hexanoate
85		ethyl 6-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)hexanoate
86		ethyl 6-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)hexanoate
87		ethyl 8-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate
88		ethyl 4-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)butanoate
89		ethyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate
90		ethyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate

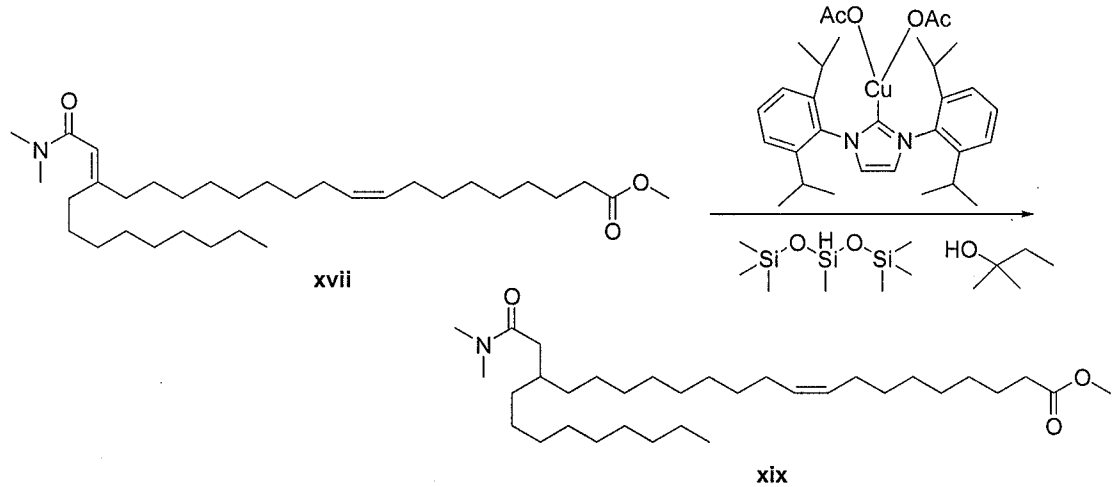
91		ethyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate
92		ethyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate
93		ethyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate
94		ethyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate
95		ethyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate
96		ethyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate

Methyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 97)



ix xvii

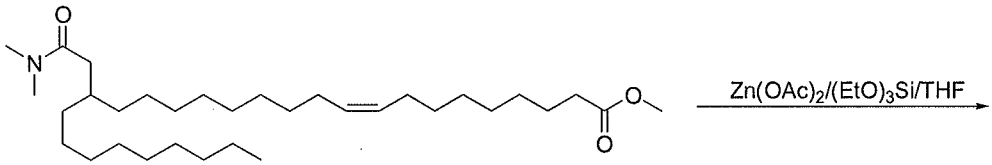
The silyl amide Peterson reagent is dissolved in THF and cooled to -63°C. To this solution is added nBuLi. The reaction is warmed to ambient temperature for 30 minutes. The ketone **ix** is dissolved in THF in a second flask. The ketone solution is transferred to the Peterson reagent over 30 minutes while maintaining the temperature between -60°C and -40°C. The reaction is warmed to -40°C for 1 hour then warmed to 0°C for 30 minutes. The reaction is quenched with sodium bicarbonate, diluted with additional water and partitioned between water/hexanes. The organics is washed with brine, dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude α,β-unsaturated amide **xvii**. The crude product is purified by flash column chromatography.



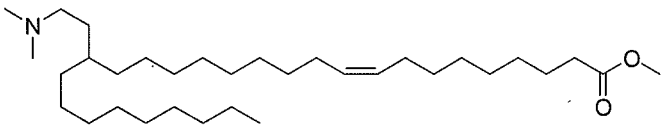
xvii xix

The Copper catalyst is dissolved in toluene under nitrogen. To this is added the PMHS in a single portion. The reaction is aged for 5 minutes. To the solutions are added the α,β-unsatured amide **xvii**. To this mixture, the t-amyl alcohol is added over 3h via syringe pump. After the addition is complete, to the solution is added 20% NH4OH to rxn in small portions. *Caution:* there is a vigorous effervescence and foaming in the beginning of the quench and it should be closely monitored and the ammonium hydroxide added slowly in small portions. The reaction is

partitioned between water and hexanes. The organics is filtered through celite and evaporated *in vacuo*. The resulting rubber solid material is pulverized using a mechanical stirrer in hexanes to give small particulates which is filtered and washed with hexanes. The organics are then evaporated *in vacuo* and purified by flash chromatography to give amide **xix**.



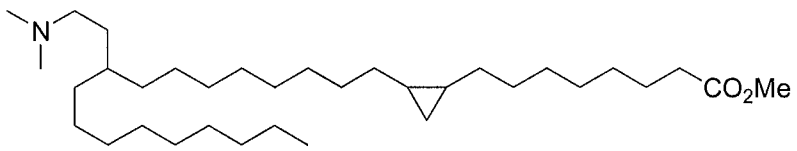
xix



97

The amide **xix** is selectively reduced by dilution in THF and treatment with zinc acetate and triethoxysilane. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon completion. The organics is dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude amine **97**. This material is purified by flash chromatography.

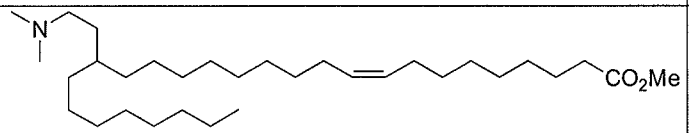
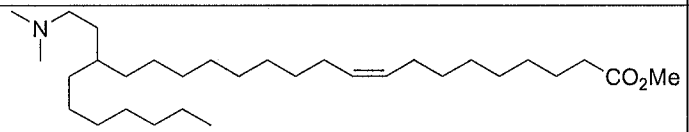
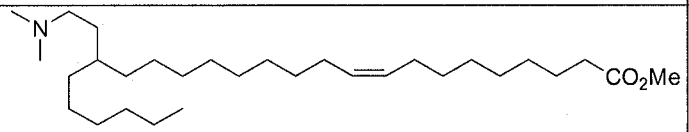
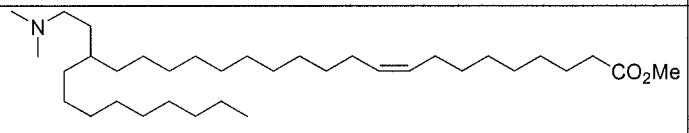
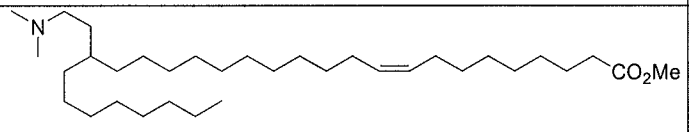
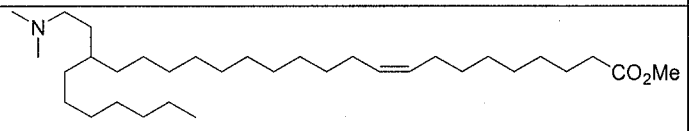
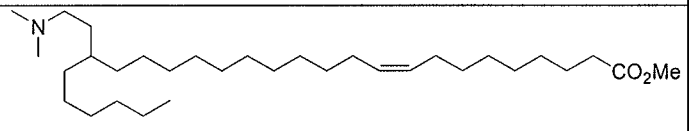
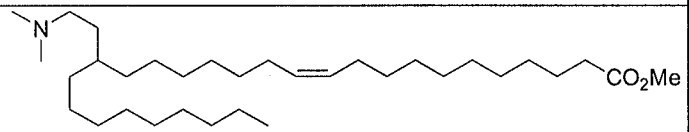
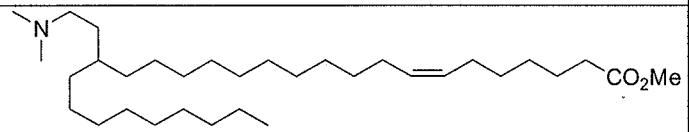
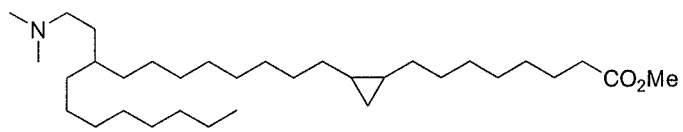
Methyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 98)

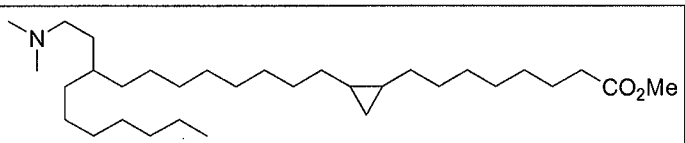
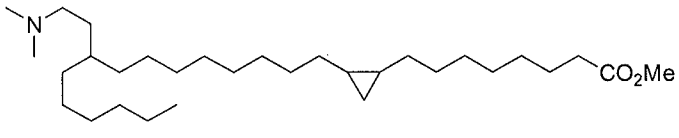
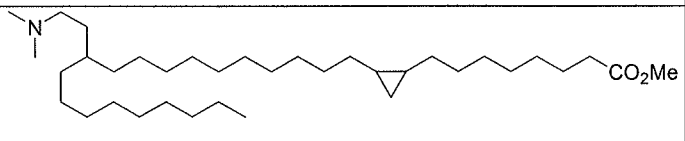
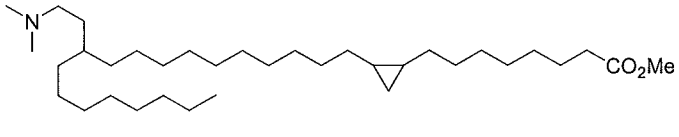
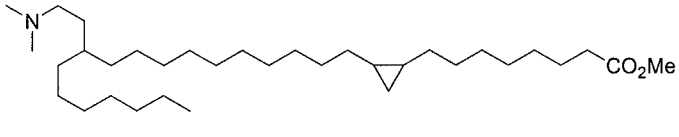
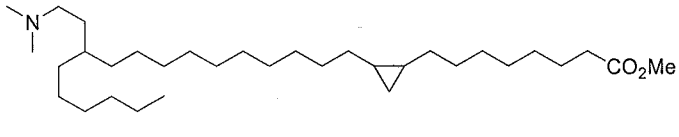
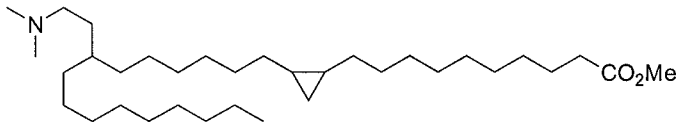
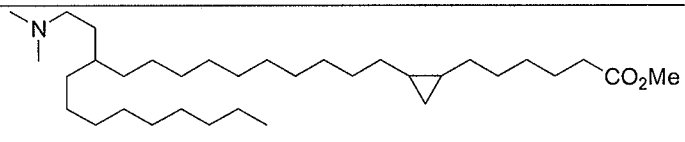
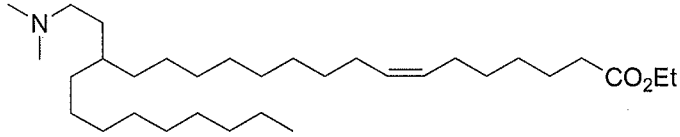


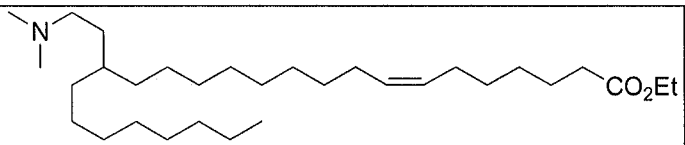
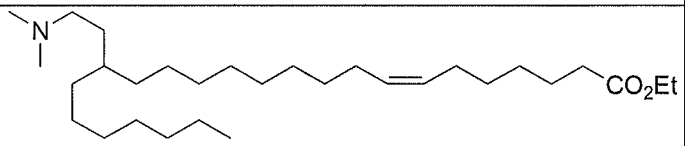
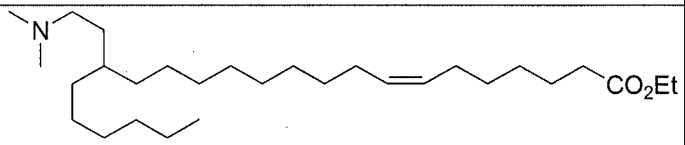
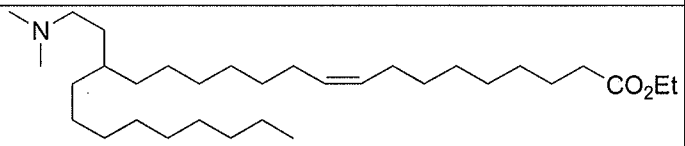
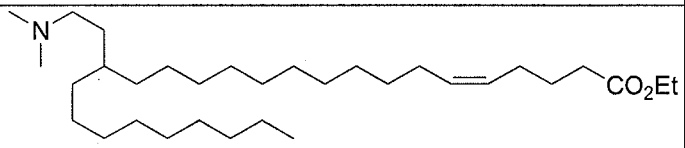
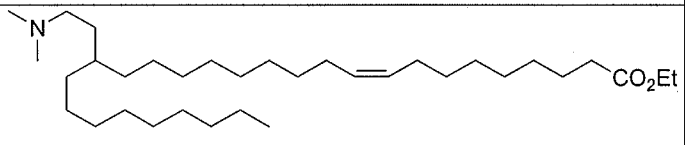
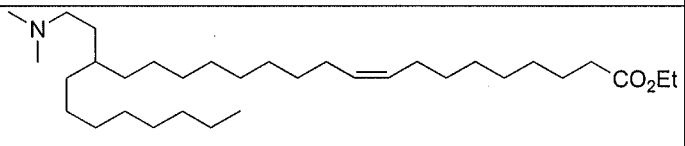
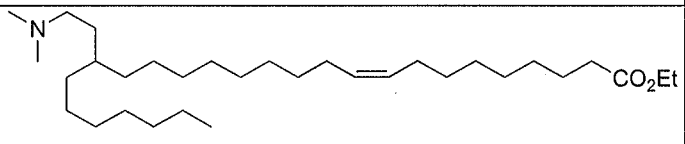
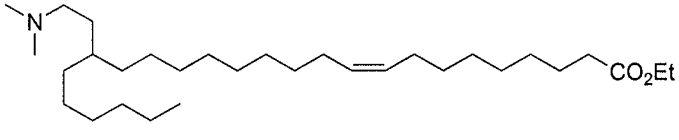
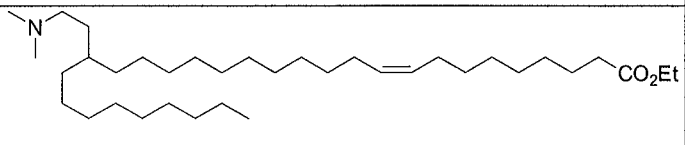
98

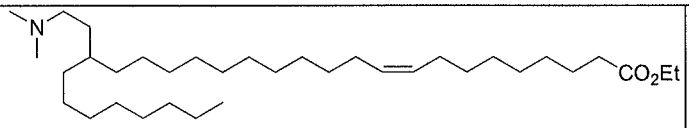
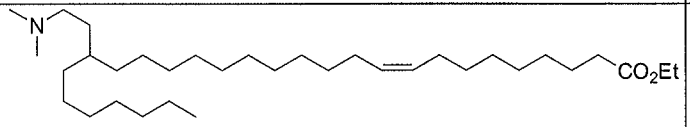
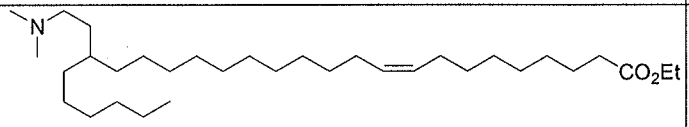
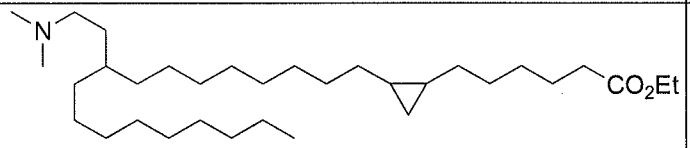
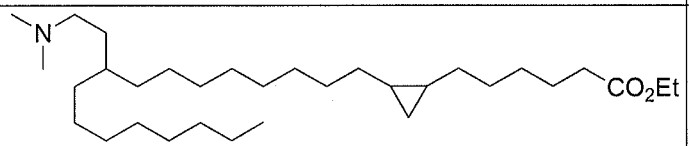
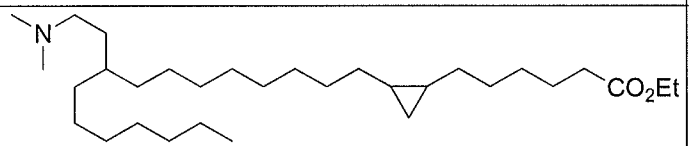
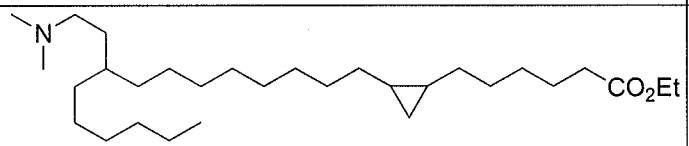
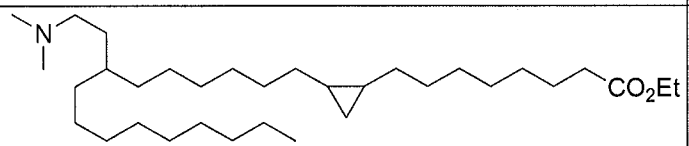
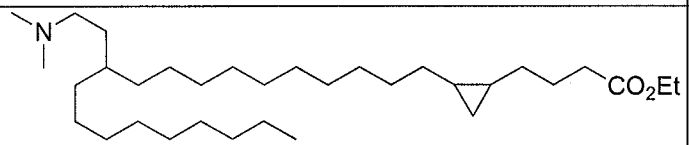
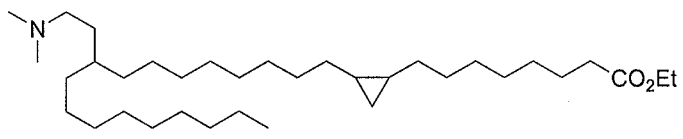
Amine **98** is prepared from ketone **x** in a manner analogous to that outlined above for compound **97**.

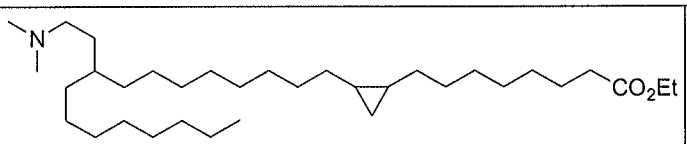
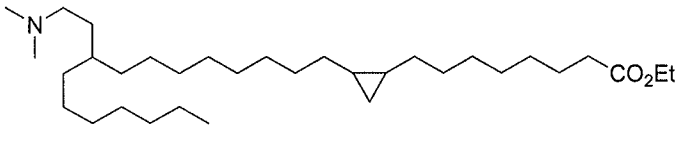
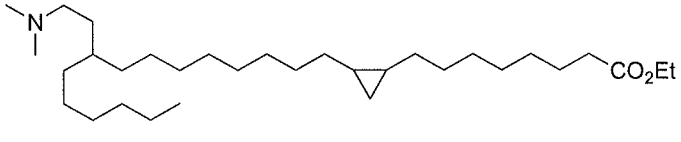
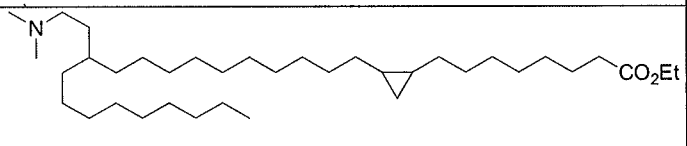
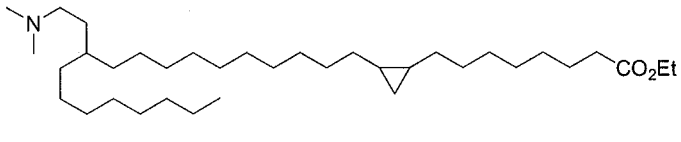
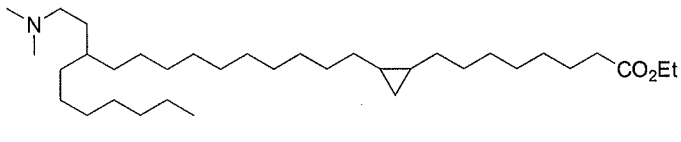
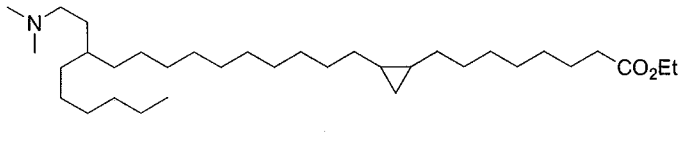
Compounds 99-144 are novel cationic lipids and are prepared according to the General Scheme 3 above.

Compound	Structure	Name
99		methyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate
100		methyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate
101		methyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate
102		methyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate
103		methyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate
104		methyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate
105		methyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate
106		methyl (11Z)-19-[2-(dimethylamino)ethyl]octacos-11-enoate
107		methyl (7Z)-19-[2-(dimethylamino)ethyl]octacos-7-enoate
108		methyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate

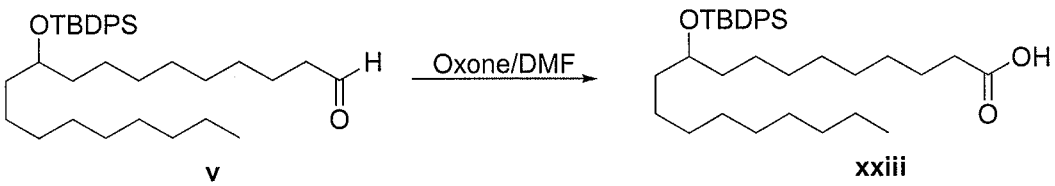
109		methyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate
110		methyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate
111		methyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate
112		methyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate
113		methyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate
114		methyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate
115		methyl 10-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)decanoate
116		methyl 6-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)hexanoate
117		ethyl (7Z)-17-[2-(dimethylamino)ethyl]hexacos-7-enoate

118		ethyl (7Z)-17-[2-(dimethylamino)ethyl]pentacos-7-enoate
119		ethyl (7Z)-17-[2-(dimethylamino)ethyl]tetracos-7-enoate
120		ethyl (7Z)-17-[2-(dimethylamino)ethyl]tricos-7-enoate
121		ethyl (9Z)-17-[2-(dimethylamino)ethyl]hexacos-9-enoate
122		ethyl (5Z)-17-[2-(dimethylamino)ethyl]hexacos-5-enoate
123		ethyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate
124		ethyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate
125		ethyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate
126		ethyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate
127		ethyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate

128		ethyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate
129		ethyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate
130		ethyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate
131		ethyl 6-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)hexanoate
132		ethyl 6-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)hexanoate
133		ethyl 6-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)hexanoate
134		ethyl 6-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)hexanoate
135		ethyl 8-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate
136		ethyl 4-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)butanoate
137		ethyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate

138		ethyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate
139		ethyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate
140		ethyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate
141		ethyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate
142		ethyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate
143		ethyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate
144		ethyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate

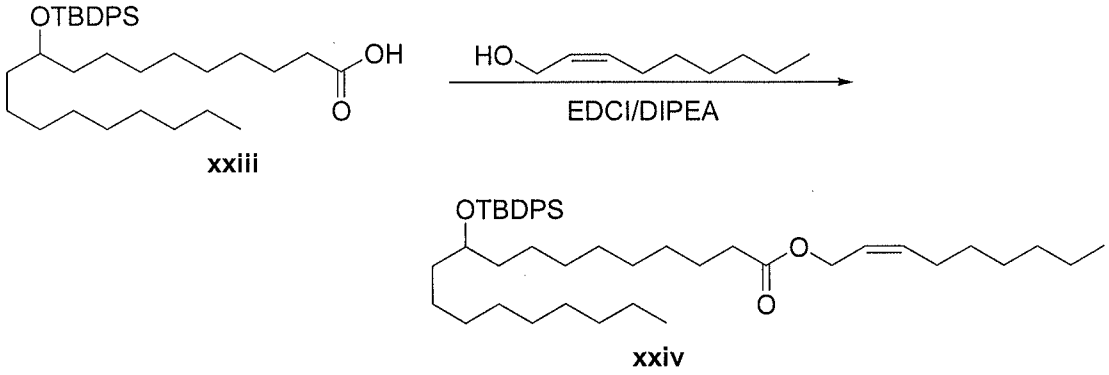
(2Z)-non-2-en-1-yl 10-(dimethylamino)nonadecanoate (Compound 145)



v **xxiii**

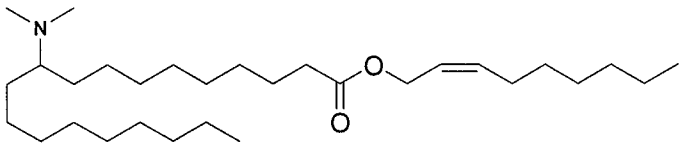
A solution of aldehyde **v** in DMF is treated with Oxone at ambient temperature. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon

completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude acid **xxiii**. This material is purified by flash chromatography.



xxiii **xxiv**

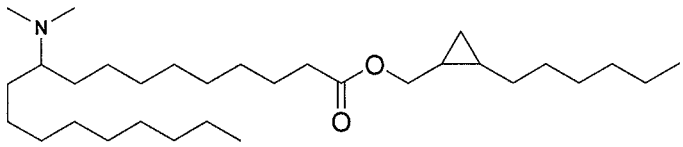
A solution of acid **xxiii** and C9-alcohol in DMF is treated with EDCI and diisopropylethylamine. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude acid **xxiv**. This material is purified by flash chromatography.



145

Conversion of silyl ether **xxiv** to Compound 145 is carried out in a manner analogous to that described for Compound 1 above

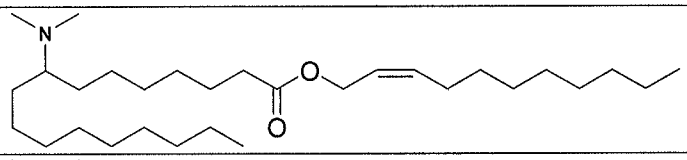
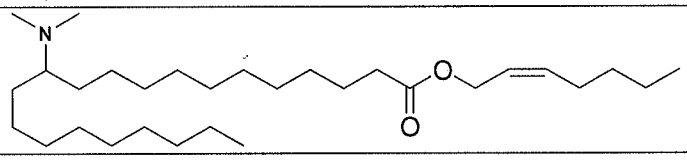
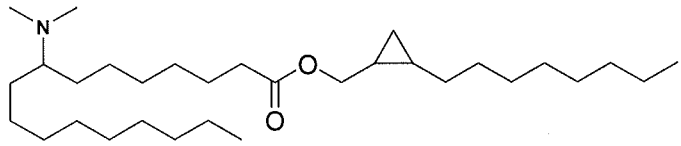
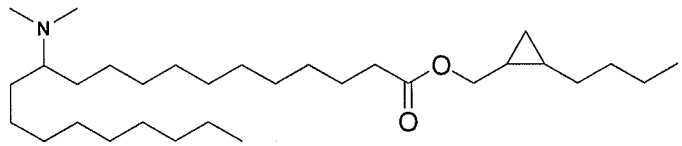
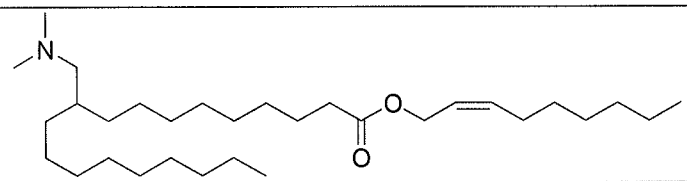
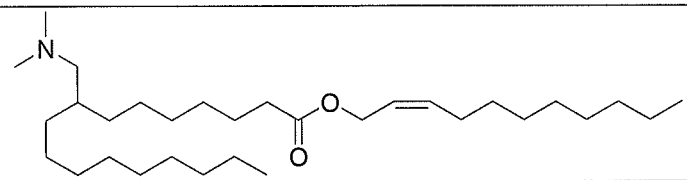
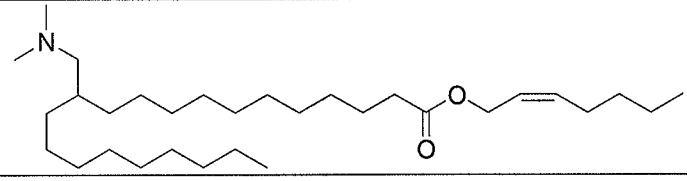
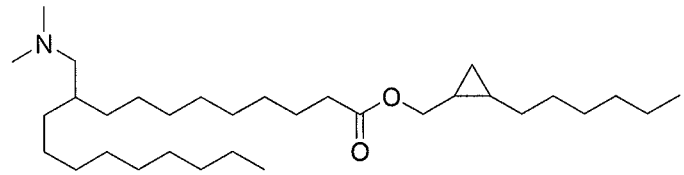
(2-hexylcyclopropyl)methyl 10-(dimethylamino)nonadecanoate (Compound 146)

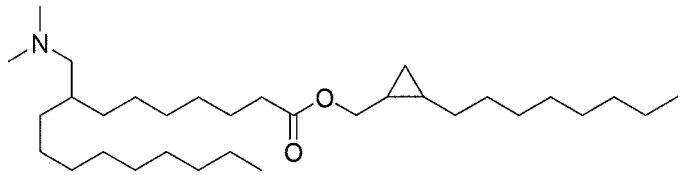
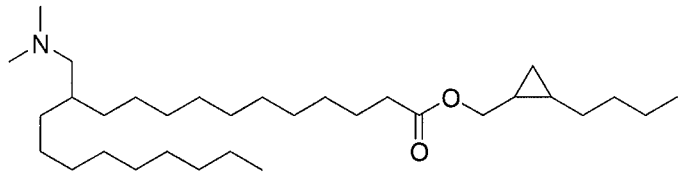
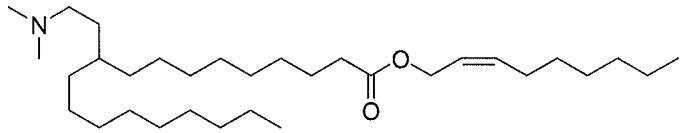
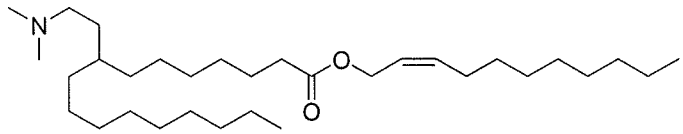
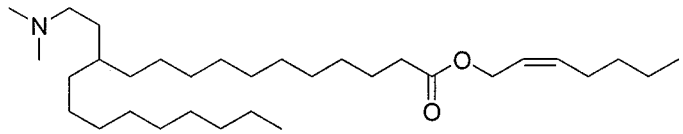
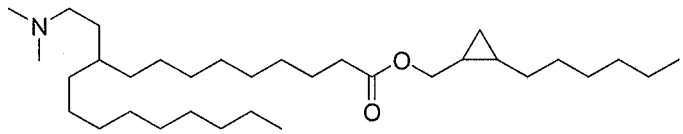
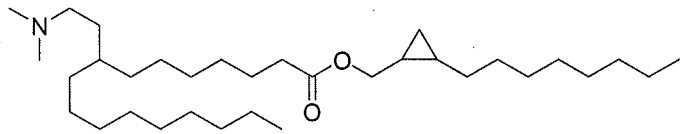
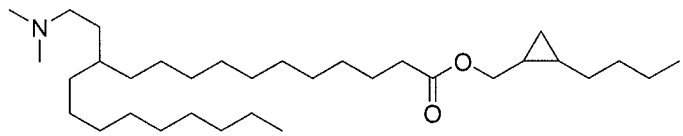


146

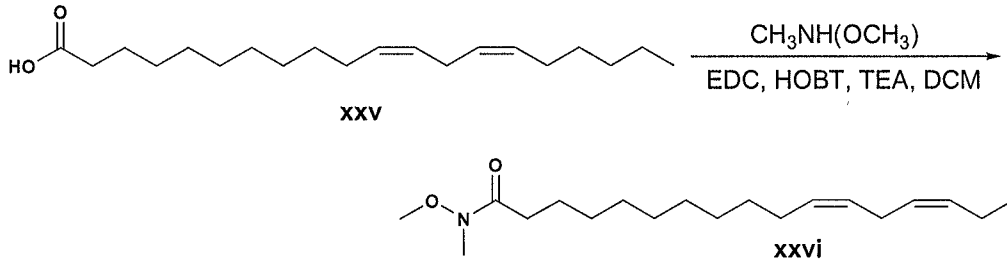
Compound 146 is prepared in a manner analogous to that described above for compound 145 employing the cyclopropanation chemistry as described for compound 2 above.

Compounds 147- 162 are novel cationic lipids and are prepared according to General Schemes 1-4 above.

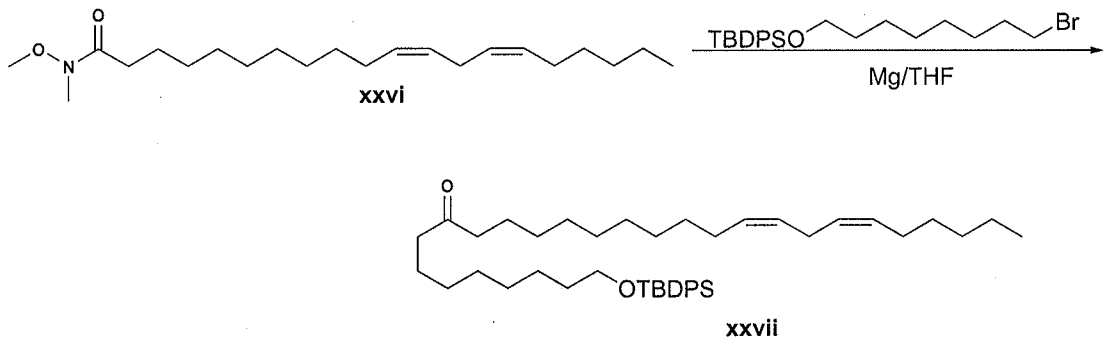
Compound	Structure	Name
147		(2Z)-undec-2-en-1-yl 8-(dimethylamino)heptadecanoate
148		(2Z)-hept-2-en-1-yl 12-(dimethylamino)henicosanoate
149		(2-octylcyclopropyl)methyl 8-(dimethylamino)heptadecanoate
150		(2-butylcyclopropyl)methyl 12-(dimethylamino)henicosanoate
151		(2Z)-non-2-en-1-yl 10-[(dimethylamino)methyl]nonadecanoate
152		(2Z)-undec-2-en-1-yl 8-[(dimethylamino)methyl]heptadecanoate
153		(2Z)-hept-2-en-1-yl 12-[(dimethylamino)methyl]henicosanoate
154		(2-hexylcyclopropyl)methyl 10-[(dimethylamino)methyl]nonadecanoate

155		(2-octylcyclopropyl)methyl 8-[(dimethylamino)methyl] heptadecanoate
156		(2-butylcyclopropyl)methyl 12-[(dimethylamino)methyl] henicosanoate
157		(2Z)-non-2-en-1-yl 10-[2-(dimethylamino)ethyl]nonadecanoate
158		(2Z)-undec-2-en-1-yl 8-[2-(dimethylamino)ethyl]heptadecanoate
159		(2Z)-hept-2-en-1-yl 12-[2-(dimethylamino)ethyl]henicosanoate
160		(2-hexylcyclopropyl)methyl 10-[2-(dimethylamino)ethyl]nonadecanoate
161		(2-octylcyclopropyl)methyl 8-[2-(dimethylamino)ethyl]heptadecanoate
162		(2-butylcyclopropyl)methyl 12-[2-(dimethylamino)ethyl]henicosanoate

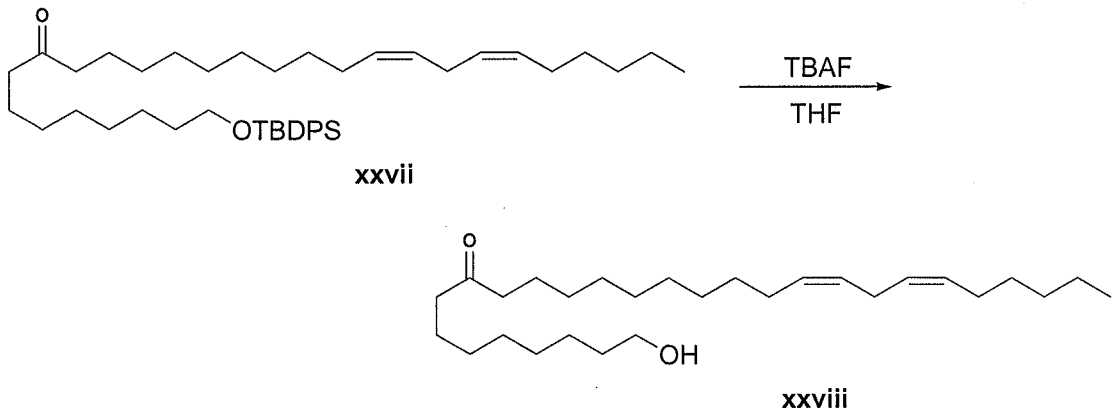
Methyl (19Z,22Z)-9-(dimethylamino)octacosa-19,22-dienoate (Compound 163)



11,14-Eicosadienoic acid, (11Z,14Z)- (50 g, 162 mmol), *N,O*-Dimethylhydroxylamine hydrochloride (31.6 g, 324 mmol), HOAt (44.1 g, 324 mmol), Et_3N (45.2 mL, 324 mmol), and EDC (62.1 g, 324 mmol) were mixed in DCM (810 mL) and stirred overnight at ambient temperature. Reaction was then washed 5 x 700 mL water, then washed 1 x 600 mL 1 M NaOH, dried with sodium sulfate, filtered through celite and evaporated to obtain 53.06 g (93%) 11,14-eicosadienamide, *N*-methoxy-*N*-methyl-, (11*Z*,14*Z*) as a clear golden oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (m, 4H), 3.68 (s, 3H), 3.18 (s, 3H), 2.77 (m, 2H), 2.41 (t, J = 7 Hz, 2H), 2.05 (m, 4H), 1.63 (m, 2H), 1.40-1.26 (m, 18H), 0.89 (t, J = 7 Hz, 3H).

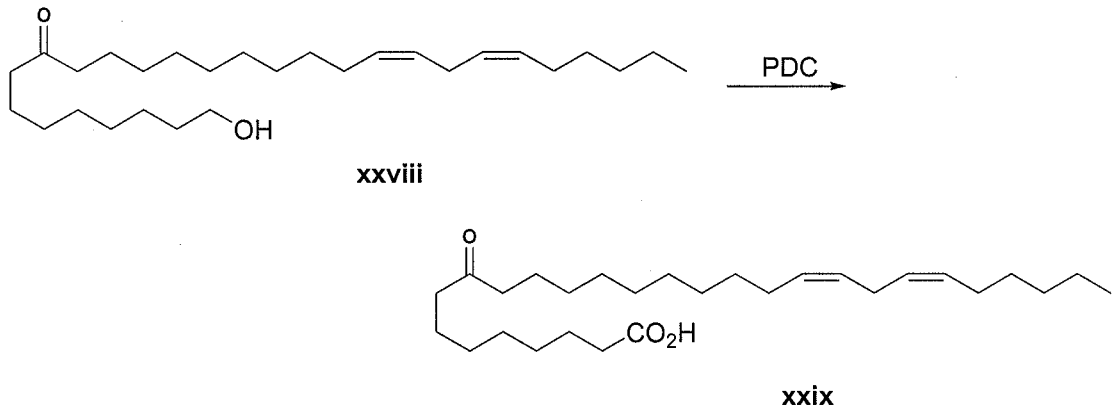


A solution of alkyl bromide in THF is treated with magnesium turnings to generate the Grignard reagent. A separate solution of Weinreb amide **xxvi** in THF is treated with this resulting Grignard solution at ambient temperature. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude ketone **xxvii**. This material is purified by flash chromatography.



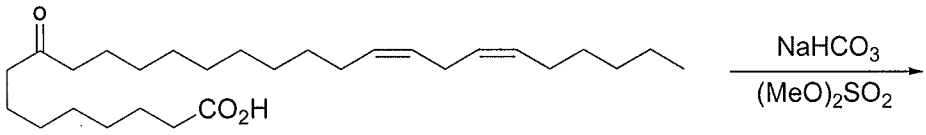
xxvii **xxviii**

A solution of silyl ether xxvii in THF is treated with TBAF. The reaction is quenched with ammonium chloride solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude alcohol **xxviii**. This material is purified by flash chromatography.

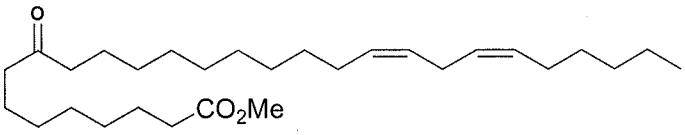


xxviii **xxix**

A solution of acohol **xxviii** in DMF is treated with pyridinium dichromate at 0 °C. The solution is warmed to ambient temperature. The reaction is quenched with water and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude acid **xxix**. This material is purified by flash chromatography.

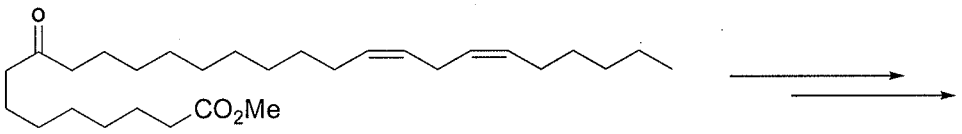


xxix

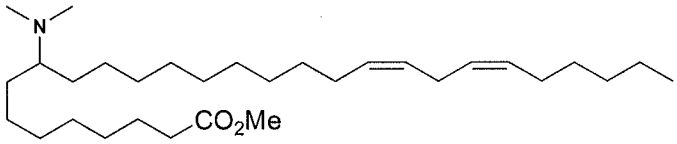


xxx

A solution of acid **xxix** in THF is treated with sodium bicarbonate and dimethylsulfate. The solution is warmed to ambient temperature. The reaction is quenched with sodium bicarbonate solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude keto-ester **xxx**. This material is purified by flash chromatography.



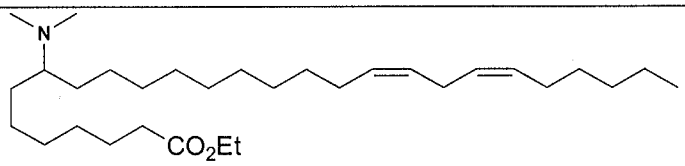
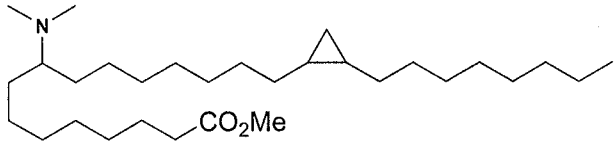
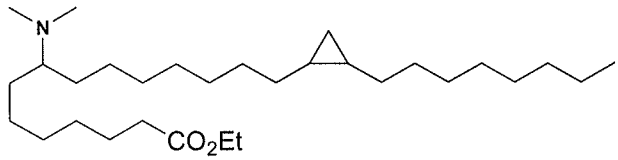
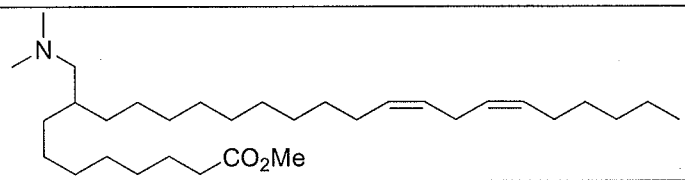
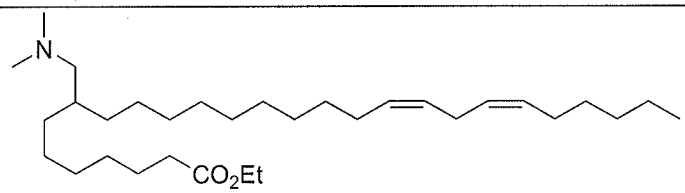
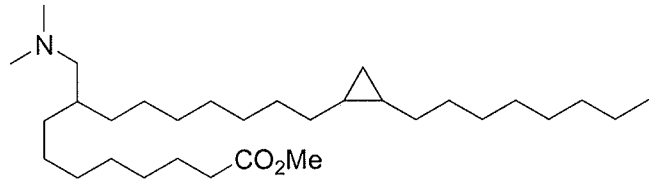
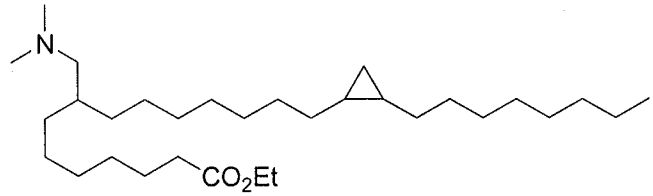
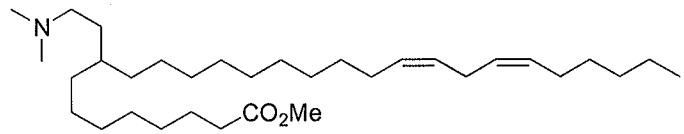
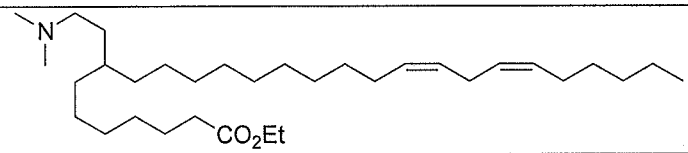
xxx

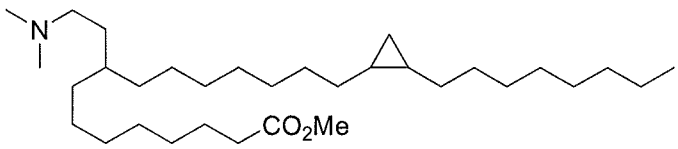
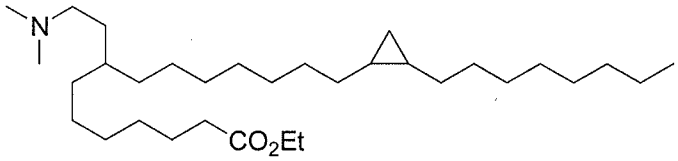


163

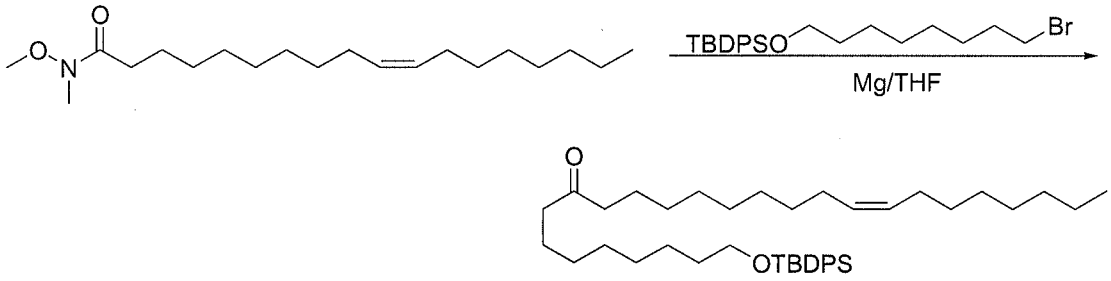
Ketone xxx is carried forward to amine **163** in a manner analogous to that described above for compound **1**.

Compounds 164-174 are novel cationic lipids and are prepared according to General Schemes 1-5 above.

Compound	Structure	Name
164		ethyl (18Z,21Z)-8-(dimethylamino)heptacosa-18,21-dienoate
165		methyl 9-(dimethylamino)-16-(2-octylcyclopropyl)hexadecanoate
166		ethyl 8-(dimethylamino)-15-(2-octylcyclopropyl)pentadecanoate
167		methyl (19Z,22Z)-9-[(dimethylamino)methyl]octacosa-19,22-dienoate
168		ethyl (18Z,21Z)-8-[(dimethylamino)methyl]heptacosa-18,21-dienoate
169		methyl 9-[(dimethylamino)methyl]-16-(2-octylcyclopropyl)hexadecanoate
170		ethyl 8-[(dimethylamino)methyl]-15-(2-octylcyclopropyl)pentadecanoate
171		methyl (19Z,22Z)-9-[2-(dimethylamino)ethyl]octacosa-19,22-dienoate
172		ethyl (18Z,21Z)-8-[2-(dimethylamino)ethyl]heptacosa-18,21-dienoate

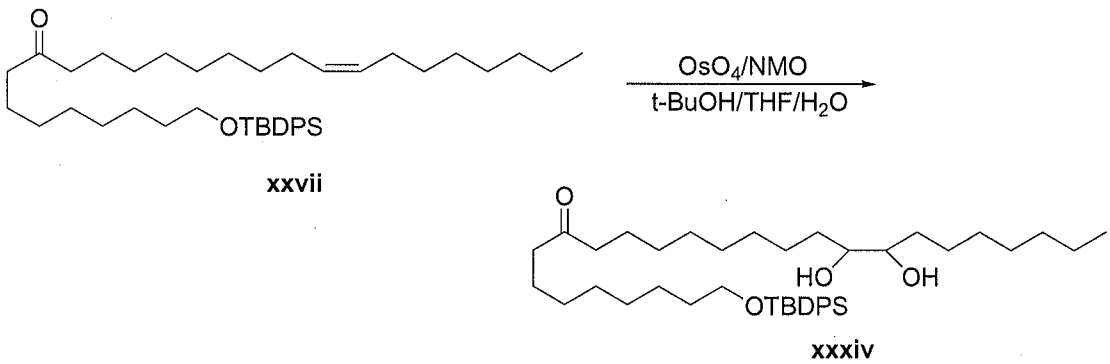
173		methyl 9-[2-(dimethylamino)ethyl]-16-(2-octylcyclopropyl)hexadecanoate
174		ethyl 8-[2-(dimethylamino)ethyl]-15-(2-octylcyclopropyl)pentadecanoate

Dimethyl (9Z)-19-(dimethylamino)heptacos-9-enedioate (Compound 175)



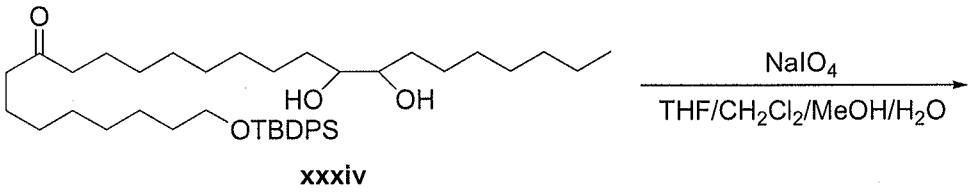
xxvii

A solution of alkyl bromide in THF is treated with magnesium turnings and aged to generate the Grignard reagent. A separate solution of Weinreb amide is treated with the Grignard reagent. The reaction is quenched with sodium bicarbonate solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude ketone **xxvii**. This material is purified by flash chromatography.

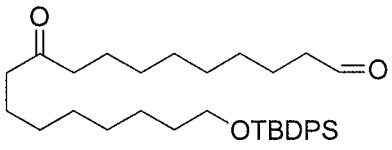


xxvii **xxxiv**

A solution of ketone in THF, tert-butanol and water is treated with osmium tetroxide and NMO. The reaction is quenched with sodium bicarbonate solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude diol **xxxiv**. This material is purified by flash chromatography.

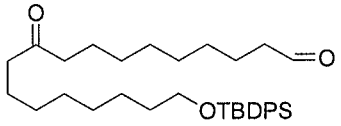


xxxiv

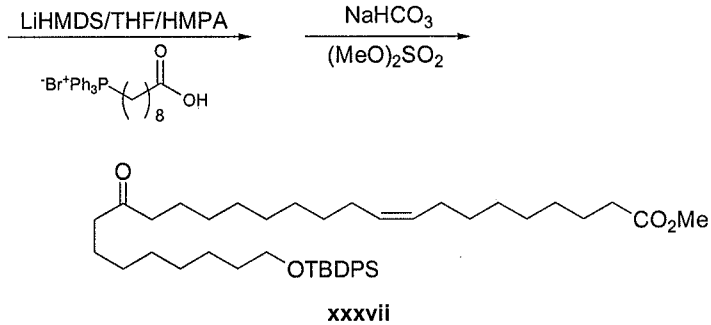


xxxv

A solution of diol **xxxiv** is taken up in THF, dichloromethane, methanol and water and treated with sodium periodate. The reaction is quenched with sodium bicarbonate solution and partitioned between hexanes and water upon completion. The organics are dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude aldehyde **xxxv**. This material is purified by flash chromatography.

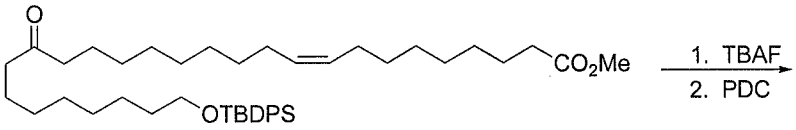


xxxv

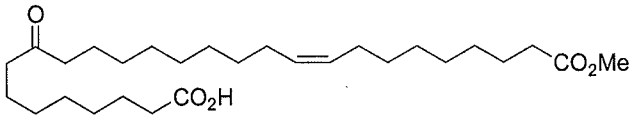


xxxvii

Ylide precursor triphenylphosphinium bromide is taken up in THF and treated with HMPA and lithium hexamethyldisilazide to generate the ylide. To this solution is added aldehyde **xxxv**. Upon reaction completion, the solution is treated with sodium bicarbonate and dimethylsulfate. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude ester **xxxvii**. The crude product is purified by flash column chromatography.



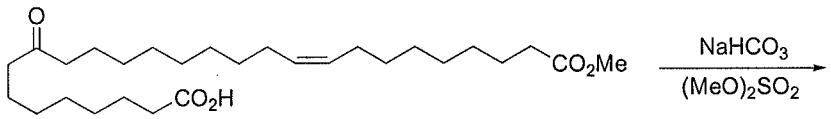
xxxvii



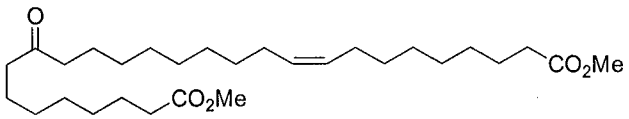
xxxviii

A solution of silyl ether **xxxvii** in THF is treated with TBAF. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude alcohol. The crude product is purified by flash column chromatography.

A solution of alcohol in DMF is treated with pyridinium dichromate. The reaction is quenched with water upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude acid **xxxviii**. The crude product is purified by flash column chromatography.

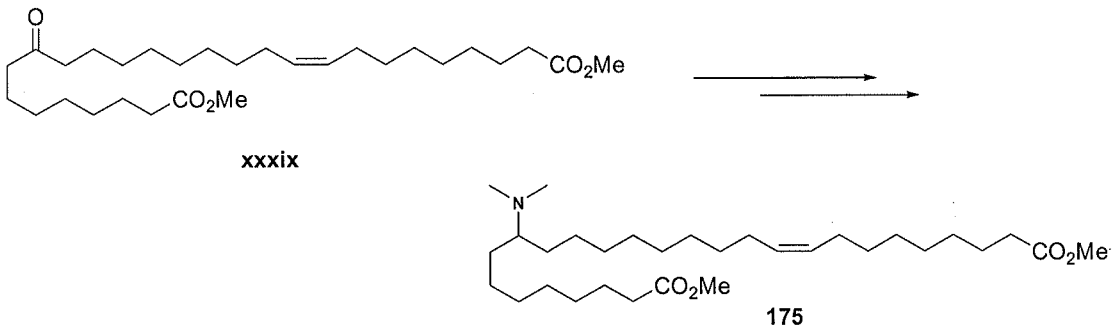


xxxviii



xxxix

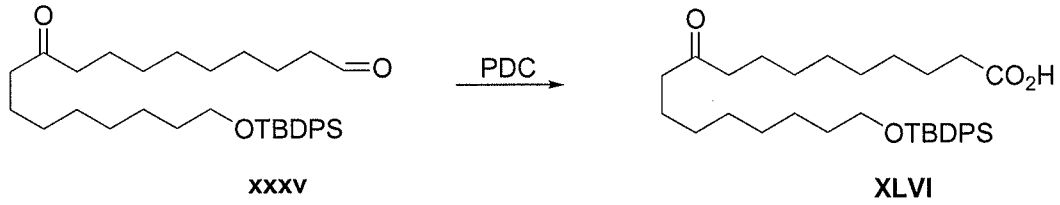
A solution of acid xxxviii in THF is treated with sodium bicarbonate and dimethyl sulfate. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude diester **xxxix**. The crude product is purified by flash column chromatography.



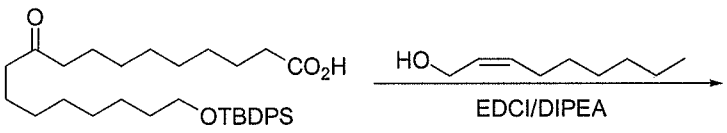
Ketone **xxxix** is converted to amine 175 in a manner analogous to that described for compound **1**.

Diesters similar to compound **175** are prepared wherein modifications to the structure are similar to those outlined in the tables above, i.e. varying lipid chain lengths, methyl and ethyl esters, inclusion of cylcopropanes, modifying position of unsaturation or cyclopropane incorporation, homologation of the dimethylamine headgroup by one or two carbons, and all possible combinations of above.

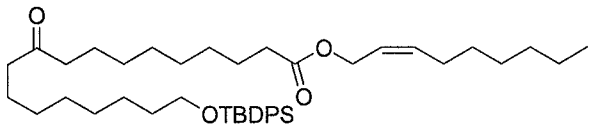
1-methyl 18-[(2Z)-non-2-en-1-yl] 9-(dimethylamino)octadecanedioate (Compound 176)



A solution of aldehyde xxxv in DMF is treated with pyridinium dichromate. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude acid **XLVI**. The crude product is purified by flash column chromatography.

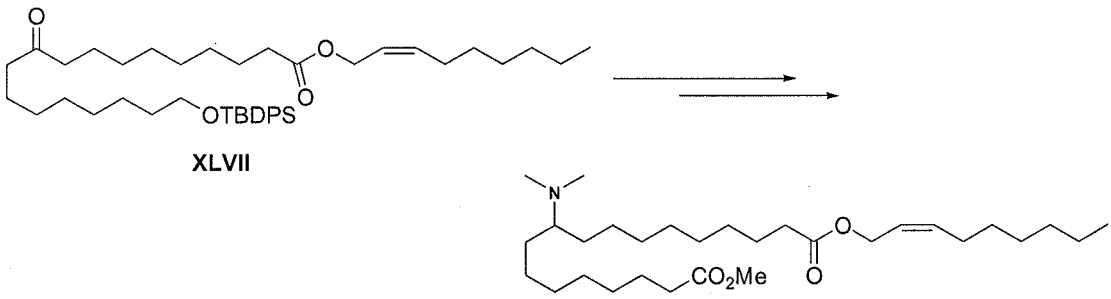


XLVI



XLVII

A solution of acid **XLVI** in dichloromethane is treated with C9 alcohol and EDCI and diisopropylethylamine. The reaction is quenched with aqueous bicarbonate solution upon completion. The reaction mixture is partitioned between water and hexanes, the organics dried over sodium sulfate, filtered and evaporated *in vacuo* to give crude keto ester **XLVII**. The crude product is purified by flash column chromatography.



XLVII

176

Ketone **XLVII** is converted to amine **176** in a manner analogous to that described for compound **175** above.

Diesters similar to compound **176** are prepared wherein modifications to the structure are similar to those outlined in the tables above, i.e. varying lipid chain lengths, methyl and ethyl esters, inclusion of cylcopropanes, modifying position of unsaturation or cyclopropane incorporation, homologation of the dimethylamine headgroup by one or two carbons, and all possible combinations of above.

LNP COMPOSITIONS

The following lipid nanoparticle compositions (LNPs) of the instant invention are useful for the delivery of oligonucleotides, specifically siRNA and miRNA:

Cationic Lipid / Cholesterol / PEG-DMG 56.6/38/5.4;

Cationic Lipid / Cholesterol / PEG-DMG 60/38/2;

Cationic Lipid/ Cholesterol / PEG-DMG 67.3/29/3.7;
Cationic Lipid / Cholesterol / PEG-DMG 49.3/47/3.7;
Cationic Lipid / Cholesterol / PEG-DMG 50.3/44.3/5.4;
Cationic Lipid / Cholesterol / PEG-C-DMA / DSPC 40/48/2/10;
Cationic Lipid / Cholesterol / PEG-DMG / DSPC 40/48/2/10; and
Cationic Lipid / Cholesterol / PEG-DMG / DSPC 58/30/2/10.

LNP process description:

The Lipid Nano-Particles (LNP) is prepared by an impinging jet process. The particles are formed by mixing lipids dissolved in alcohol with siRNA dissolved in a citrate buffer. The mixing ratio of lipids to siRNA are targeted at 45-55% lipid and 65-45% siRNA. The lipid solution can contain a novel cationic lipid of the instant invention, a helper lipid (cholesterol) , PEG (e.g. PEG-C-DMA, PEG-DMG) lipid, and DSPC at a concentration of 5-15 mg/mL with a target of 9-12 mg/mL in an alcohol (for example ethanol). The ratio of the lipids can have a mole percent range of 25-98 for the cationic lipid with a target of 35-65, the helper lipid can have a mole percent range from 0-75 with a target of 30-50, the PEG lipid can have a mole percent range from 1-15 with a target of 1-6, and the DSPC can have a mole precent range of 0-15 with a target of 0-12. The siRNA solution can contain one or more siRNA sequences at a concentration range from 0.3 to 1 .0 mg/mL with a target of 0.3 -0.9 mg/mL in a sodium citrate buffered salt solution with pH in the range of 3.5-5. The two liquids are heated to a temperature in the range of 15-40°C, targeting 30-40°C, and then mixed in an impinging jet mixer instantly forming the LNP. The teeID can have a range from 0.25 to 1.0 mm and a total flow rate from 10 -600 mL/min. The combination of flow rate and tubing ID can have the effect of controlling the particle size of the LNPs between 30 and 200 nm. The solution can then be mixed with a buffered solution at a higher pH with a mixing ratio in the range of 1:1 to 1:3 vol:vol but targeting 1:2 vol:vol. This buffered solution is at a temperature in the range of 15-40°C, targeting 30-40°C. The mixed LNPs are held from 30 minutes to 2 hrs prior to an anion exchange filtration step. The temperature during incubating is in the range of 15-40°C, targeting 30-40°C. After incubating the solution is filtered through a 0.8 um filter containing an anion exchange separation step. This process can use tubing IDs ranging from 1 mm ID to 5 mm ID and a flow rate from 10 to 2000 mL/min. The LNPs are concentrated and diafiltered via an ultrafiltration process where the alcohol is removed and the citrate buffer is exchanged for the final buffer solution such as phosphate buffered saline. The ultrafiltration process can use a

tangential flow filtration format (TFF). This process can use a membrane nominal molecular weight cutoff range from 30 -500 KD. The membrane format is hollow fiber or flat sheet cassette. The TFF processes with the proper molecular weight cutoff can retain the LNP in the retentate and the filtrate or permeate contains the alcohol; citrate buffer; final buffer wastes. The TFF process is a multiple step process with an initial concentration to a siRNA concentration of 1 -3 mg/mL. Following concentration, the LNPs solution is diafiltered against the final buffer for 10 -20 volumes to remove the alcohol and perform buffer exchange. The material can then be concentrated an additional 1-3 fold. The final steps of the LNP process are to sterile filter the concentrated LNP solution and vial the product.

Analytical Procedure:

1) siRNA concentration

The siRNA duplex concentrations are determined by Strong Anion-Exchange High-Performance Liquid Chromatography (SAX-HPLC) using Waters 2695 Alliance system (Water Corporation, Milford MA) with a 2996 PDA detector. The LNPs, otherwise referred to as RNAi Delivery Vehicles (RDVs), are treated with 0.5% Triton X-100 to free total siRNA and analyzed by SAX separation using a Dionex BioLC DNAPac PA 200 (4 × 250 mm) column with UV detection at 254 nm. Mobile phase is composed of A: 25 mM $NaClO_4$, 10 mM Tris, 20% EtOH, pH 7.0 and B: 250 mM $NaClO_4$, 10 mM Tris, 20% EtOH, pH 7.0 with liner gradient from 0-15 min and flow rate of 1 ml/min. The siRNA amount is determined by comparing to the siRNA standard curve.

2) Encapsulation rate

Fluorescence reagent SYBR Gold is employed for RNA quantitation to monitor the encapsulation rate of RDVs. RDVs with or without Triton X-100 are used to determine the free siRNA and total siRNA amount. The assay is performed using a SpectraMax M5*e* microplate spectrophotometer from Molecular Devices (Sunnyvale, CA). Samples are excited at 485 nm and fluorescence emission is measured at 530 nm. The siRNA amount is determined by comparing to the siRNA standard curve.

Encapsulation rate = (1- free siRNA/total siRNA) ×100%

3) Particle size and polydispersity

RDVs containing 1 μg siRNA are diluted to a final volume of 3 ml with 1 × PBS. The particle size and polydispersity of the samples is measured by a dynamic light scattering method using ZetaPALS instrument (Brookhaven Instruments Corporation, Holtsville, NY). The scattered intensity is measured with He–Ne laser at 25°C with a scattering angle of 90°.

4) Zeta Potential analysis

RDVs containing 1 μg siRNA are diluted to a final volume of 2 ml with 1 mM Tris buffer (pH 7.4). Electrophoretic mobility of samples is determined using ZetaPALS instrument (Brookhaven Instruments Corporation, Holtsville, NY) with electrode and He–Ne laser as a light source. The Smoluchowski limit is assumed in the calculation of zeta potentials.

5) Lipid analysis

Individual lipid concentrations is determined by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) using Waters 2695 Alliance system (Water Corporation, Milford MA) with a Corona charged aerosol detector (CAD) (ESA Biosciences, Inc, Chelmsford, MA). Individual lipids in RDVs are analyzed using an Agilent Zorbax SB-C18 (50 × 4.6 mm, 1.8 μm particle size) column with CAD at 60 °C. The mobile phase is composed of A: 0.1% TFA in H_2O and B: 0.1% TFA in IPA. The gradient can change from 60% mobile phase A and 40% mobile phase B from time 0 to 40% mobile phase A and 60% mobile phase B at 1.00 min; 40% mobile phase A and 60% mobile phase B from 1.00 to 5.00 min; 40% mobile phase A and 60% mobile phase B from 5.00 min to 25% mobile phase A and 75% mobile phase B at 10.00 min; 25% mobile phase A and 75% mobile phase B from 10.00 min to 5% mobile phase A and 95% mobile phase B at 15.00 min; and 5% mobile phase A and 95% mobile phase B from 15.00 to 60% mobile phase A and 40% mobile phase B at 20.00 min with flow rate of 1 ml/min. The individual lipid concentration is determined by comparing to the standard curve with all the lipid components in the RDVs with a quadratic curve fit. The molar percentage of each lipid is calculated based on its molecular weight.

Utilizing the above described LNP process, specific LNPs with the following ratios are identified:

Nominal composition:

Cationic Lipid / Cholesterol / PEG-DMG 60/38/2

Cationic Lipid / Cholesterol / PEG-DMG / DSPC 58/30/2/10

Luc siRNA

5'-iB-**A***U***AAGG***C***U***A***U**GAAGAGA*U*ATT**-iB 3' (SEQ.ID.NO.:1)

3'-UU*U***A***U***U***C***C**GA*U***A***C***U***U***C***U***C***U*AU-5' (SEQ.ID.NO.:2)

AUGC – Ribose

iB – Inverted deoxy abasic

UC – 2' Fluoro

AGT – 2' Deoxy

AGU – 2' OCH_3

Nominal composition

Cationic Lipid /Cholesterol/PEG-DMG 60/38/2

Cationic Lipid / Cholesterol / PEG-DMG / DSPC 40/48/2/10

Cationic Lipid / Cholesterol / PEG-DMG / DSPC 58/30/2/10

ApoB siRNA

5'-iB-CUUU*AACAA*UUCCU*GAAA*U**TsT**-iB-3' (SEQ ID NO.:3)

3'-UsU*GAAAUUGUUAAGGA*CUsUsUsA-5' (SEQ ID NO.:4)

AUGC – Ribose

iB – Inverted deoxy abasic

UC – 2' Fluoro

AGT – 2' Deoxy

AGU – 2' OCH_3

UsA – phophorothioate linkage

beta-catenin siRNA

5'-iB-CUGUUGGAUUGAUUCGAAAUsU-iB-3' (SEQ ID NO.:5)

3'-UsUG*ACAACCUAACUA*AG*CUUU*-5' (SEQ ID NO.:6)

AUGC – Ribose

iB – Inverted deoxy abasic

UC – 2' Fluoro

AGT – 2' Deoxy

AGU – 2' OCH_3

UsA – phophorothioate linkage

5'-iB-*A***C***GA***C****UA****G****UU****C****AGUU****G****C****U****UUsU-iB-3' (SEQ ID NO.:7)

3'-UsUUGCUG*AUC*AAGU*C*A*A*CG*AA*-5' (SEQ ID NO.:8)

AUGC – Ribose

iB – Inverted deoxy abasic

UC – 2' Fluoro

AGT – 2' Deoxy

AGU – 2' OCH_3

UsA – phophorothioate linkage

5'-iB-*AC*G*AC*U**A**G*UU*C**AGU**U**G**C**UUUU**-iB-3' (SEQ ID NO.:9)

3'-UUUGCUG*AUC*AAGUC*A**A**C*G*AA*-5' (SEQ ID NO.:10)

AUGC – Ribose

iB – Inverted deoxy abasic

UC – 2' Fluoro

AGT – 2' Deoxy

AGU – 2' OCH_3

UsA – phophorothioate linkage

Oligonucleotide synthesis is well known in the art. (See US patent applications: US 2006/0083780, US 2006/0240554, US 2008/0020058, US 2009/0263407 and US 2009/0285881 and PCT patent applications: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405 and WO2010/054406). The siRNAs disclosed and utilized in the Examples are synthesized via standard solid phase procedures.

EXAMPLE 1

Mouse In Vivo Evaluation of Efficacy

LNPs utilizing Compounds 1-176, in the nominal compositions described immediately above, are evaluated for *in vivo* efficacy. The siRNA can target the mRNA transcript for the firefly (Photinus pyralis) luciferase gene (Accession # M15077). The primary sequence and chemical modification pattern of the luciferase siRNA is displayed above. The *in vivo* luciferase model employs a transgenic mouse in which the firefly luciferase coding sequence is present in all cells. ROSA26- LoxP-Stop-LoxP-Luc (LSL-Luc) transgenic mice licensed from the Dana Farber Cancer Institute are induced to express the Luciferase gene by first removing the LSL sequence with a recombinant Ad-Cre virus (Vector Biolabs). Due to the organo-tropic nature of the virus, expression is limited to the liver when delivered via tail vein injection. Luciferase expression levels in liver are quantitated by measuring light output, using an IVIS imager (Xenogen) following administration of the luciferin substrate (Caliper Life Sciences). Pre-dose luminescence levels is measured prior to administration of the RDVs.

Luciferin in PBS (15mg/mL) is intraperitoneally (IP) injected in a volume of 150 μL. After a four minute incubation period mice are anesthetized with isoflurane and placed in the IVIS imager. The RDVs (containing siRNA) in PBS vehicle are tail vein injected in a volume of 0.2 mL. Final dose levels can range from 0.1 to 0.5 mg/kg siRNA. PBS vehicle alone is dosed as a control. Mice are imaged 48 hours post dose using the method described above. Changes in luciferin light output directly correlate with luciferase mRNA levels and represent an indirect measure of luciferase siRNA activity. In vivo efficacy results are expressed as % inhibition of luminescence relative to pre-dose luminescence levels.

EXAMPLE 2

In vitro ApoE binding assay

LNPs are incubated at 37°C in 90% rhesus serum at a final LNP concentration of 4ug/mL. Incubation is for 20 minutes with orbital rotation. After incubation, the samples are diluted 1:20 in PBS and 100 μL of each diluted sample is aliquoted to wells of an anti-PEG antibody coated 96-well plate (Life Diagnostics Cat. No. P-0001PL. After incubation at room temperature for 1 hour, the plate is washed 5X with 300uL PBS. After washing, 50uL of 0.2% Triton X-100 is added to each well and the plate incubated at 37°C for 10 minutes, followed by shaking on a plate shaker for 1 minute at 750 rpm. Samples are frozen prior to performing the ApoE ELISA and stem loop PCR analysis of samples.

An ApoE ELISA assay is performed to quantitate ApoE bound to the LNPs after incubation in rhesus serum. Anti-ApoE antibody (Milipore, Cat No. AB947) is diluted 1:1000 in PBS and 100 μL of diluted antibody is added to each well of a polystyrene high binding plate. The plate with antibody is incubated overnight at 4°C, after which the plate is washed 2X with 200μL of PBS. Next, 200μL of buffer containing 1% BSA and 0.05% Tween-20 in PBS (Incubation Buffer) is added to each well followed by incubation at room temperature for 1 hour. Plates are washed 5X with PBS containing 0.05% Tween-20. Frozen Triton lysis test samples are thawed and diluted 1:6 with incubation buffer and 100 μL of test sample is aliquoted to wells of the ApoE antibody plate. Incubation is for 1 hour at room temperature followed by a 5X wash with PBS containing 0.05% Tween-20. After washing, 100μL of biotinylated anti-ApoE antibody (Mabtech, Cat. ANo. E887-biotin), diluted 1:500 in incubation buffer, is added to each well and incubated for 1 hour at room temperature, followed by a 5X wash with 0.05% Tween-20 in PBS. 100μL per well, of Streptavidin-HPR (Thermo, Cat. No.

TS-125-HR), is then added and incubated for 1 hour at room temperature. After washing 5X with 0.05% Tween-20 in PBS, 100µL of TMB Substrate (Thermo, Cat. No. 34028) is added to each well, followed by incubation at room temperature for 20 minutes in the dark. The colorimetric reaction is stopped with 100µL of TMB Stop Solution (KPL, Cat. No. 50-85-04) and absorbance at 450nm is determined. An ApoE standard curve is prepared by diluting rhesus Recombinant ApoE in incubation buffer with 0.03% Triton X-100 with concentrations ranging from 100 ng/mL to 0.78 ng/mL. ApoE standards are evaluated in the ELISA in parallel to the test samples. A rhesus serum only (no LNP) control is utilized to obtain a background subtraction for non-LNP dependent ApoE signal in the ELISA.

Stem Loop RT-PCR Protocol

To normalize to the ApoE bound to the amount of LNP bound to the anti-PEG antibody plate, the amount of siRNA retained in the anti-PEG antibody well is quantitated by stem-loop PCR and related to the number of siRNAs encapsulated per LNP, to give an approximate measure of total LNP particles bound per well.

Preparation of the Spiked Standard Curve Samples:

The standard curve is prepared using the molecular weight of the siRNA (13693 g/mol for ApoB 17063) to calculate the copy number. The high standard should contain 10^{11} copies per 3µl. A 10-fold serial dilution is performed across a row of an assay plate until the lowest standard contains 10^2 copies per 3µl. One could dilute 0.2% Triton X-100 1:80 in water and pipette 20 µL of the diluted Triton X-100 into 10 wells of a 96 well plate. 30µL of the serial diluted standard curve and mix is added to each well of the plate. 10 µL of the spiked standard curve is used in the reverse transcription reaction.

Stem-Loop RT-PCR – test samples and standard curve:

Triton lysates from the PEG antibody plate capture is diluted 1 to 2000 in nuclease free water. 10µL of 'RT-Primer Mix' (Applied Biosystem's TaqMan MicroRNA Reverse Transcription Kit Cat. No. 4366596) is added to each well of a 96-well Micro-Amp QPCR plate (ABI Cat# N801-0560).

RT Primer Mix Components	**µL / rxn**	**Final conc.**

ApoB RT-primer (10uM)	0.6	200 nM
10x buffer	2	
Water	7.4	

ApoB RT primer sequence: 5' GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGAC**CTTTAACA3'**(SEQ.ID.NO.:11)

10μL of each test sample (diluted 1 to 2000) or spiked standard curve (above) is aliquoted into the 96-well plate. The plate is covered with a mat (ABI Cat. No. N801-0550), to minimize evaporation. The plate is briefly centrifuged at 800 rpm for 1 minute. Next, the plate is run on a thermocycler using the following cycling parameters:

Cycling:	94°C	10 minutes
	75°C	2 minutes
	60°C	3 minutes
	50°C	3 minutes
	40°C	3 minutes
	30°C	3 minutes
	4°C	hold

Next, 10μL of 'RT Mix' is added to each well (Applied Biosystem's TaqMan MicroRNA Reverse Transcription Kit Cat. No. 4366596)

RT Mix Components	**μL / rxn**
100 mM dNTP	0.3
10x RT buffer	1
Rnase Inhibitor	0.38
Multiscribe RT enzyme	1
Water	7.32

The RT cycling reaction is composed of 10μL test sample, 10μL of RT primer mix and 10 μL of RT Mix components for a total volume of 30μL. The final concentration of the RT-primer in the total 30 μL total RT mix is 200nM. The plate is then sealed with the same

plate mat, briefly centrifuged at 800 rpm for 1 minute, then run on the thermocycler using the following cycling parameters:

Cycling:	16°C	30 minutes
	42°C	30 minutes
	85°C	5 minutes
	4°C	hold

Next, 15 µL of Fast Enzyme / primer-probe mix is added to each well of a new Fast 96-well plate (Applied Biosystem's TaqMan Fast Universal PCR Master Mix, Cat. No. 4352042)

ApoB		
PCR Master Mix Components	**µL / rxn**	**Final Conc.**
Fast Enyzme Mix (2x stock)	10	
forward primer (100uM)	0.18	**900 nM**
reverse primer (100uM)	0.18	**900 nM**
probe (10uM)	0.05	**250 nM**
Water	4.59	

ApoB primers and probe sequence:

17063DC F3 GGCGCGAAATTTCAGGAATTGT (SEQ.ID.NO.:12)

17063DC Pr2 CACTGGATACGACCTTTAACA (SEQ.ID.NO.:13)

Universal R2 AGTGCAGGGTCCGAG (SEQ.ID.NO.:14)

5 µL of each RT reaction is added to the Fast Enzyme Mix plate. The plate is centrifuged for 1 minute at 1000 rpm and the QPCR analysis is performed on an ABI7900 with Fast Block. Cycling parameters is: 1 cycle - 95°C for 20 seconds, followed by 40 Cycles - 95°C for 1 seconds, 60°C for 20 seconds.

The QPCR result is utilized to calculate the siRNA concentration in the PEG antibody capture plate Triton lysates. Based on an estimate of 500 siRNA per LNP particle, the

number of LNPs retained in each well of the anti-PEG antibody plate can be calculated. Using the ApoE concentration per well, as determined by the ApoE ELISA and the number of LNP particles per well, an approximate ApoE molecules bound per LNP particle can be calculated.

EXAMPLE 3

Heparin Sepharose HI-TRAP™ Binding Assay

Lipid nanoparticles (LNP) with neutral surface charge are not retained after injection onto heparin sepharose with 1X Dulbecco's phosphate buffered saline (DPBS) as the running buffer but elute in the column void volume. Serum apolipoprotein E (ApoE) exhibits high affinity binding with heparin sulfate and it can be shown that LNPs bind to heparin sepharose to an extent dependent on their intrinsic ability to bind ApoE (depending on both lipid nanoparticle composition and ApoE concentration) after incubation with purified and/or recombinant human ApoE or serum samples. Lipid nanoparticles with surface bound ApoE bind to heparin sepharose with high affinity can be eluted only at high salt (1M NaCl).

A heparin sepharose binding assay is developed to assess serum ApoE binding to lipid nanoparticles based on the high affinity interaction that ApoE-LNP complexes exhibit toward heparin sepharose.

Incubations

Lipid nanoparticles are incubated at 37°C for 20 min at a final siRNA concentration of 50 μg/mL with various concentrations of either purified or recombinant human apolipoprotein E or 0.1-50% rat/mouse/rhesus monkey/human serum in 1X Dulbecco's phosphate buffered saline (DPBS). After incubation with ApoE or serum LNP samples are diluted 10-fold using 1X DPBS and analyzed by heparin sepharose chromatography. Peak area of retained LNP (after subtraction of appropriate blank signals) is compared to total peak area of LNP control without ApoE and/or serum incubation to determine the percentage of the LNP which undergoes shift to high affinity heparin interaction after incubation with ApoE/serum.

Heparin Sepharose HI-TRAP™ Chromatographic Conditions

A heparin sepharose HI-TRAP™ chromatography column (GE Healthcare; 1 mL bed volume) is equilibrated with either 1X or 2X Dulbecco's PBS; the higher 2X salt

concentration is used for LNPs with higher intrinsic retention on heparin sepharose (presumably due to higher positive surface charge).

Mobile Phase A: 1X or 2X DPBS

Mobile Phase B: 1M NaCl in 10 mM sodium phosphate buffer, pH 7.0

100% A delivered isocratically for 10 min followed by step gradient to 100% B; hold for additional 10 min; step gradient back to 100% A and reequilibrate for additional 10 min prior to injection of next sample

Flow rate: 1 mL/min

Sample injection volume: 50 μL.

Detection: UV @260 nm

EXAMPLE 4

Rat *In Vivo* Evaluation of Efficacy and Toxicity

LNPs utilizing compounds in the nominal compositions described above, are evaluated for in vivo efficacy and increases in alanine amino transferase and aspartate amino transferase in Sprague-Dawley (Crl:CD(SD) female rats (Charles River Labs). The siRNA targets the mRNA transcript for the ApoB gene (Accession # NM 019287). The primary sequence and chemical modification pattern of the ApoB siRNA is displayed above. The RDVs (containing siRNA) in PBS vehicle are tail vein injected in a volume of 1 to 1.5 mL. Infusion rate is approximately 3 ml/min. Five rats are used in each dosing group. After LNP administration, rats are placed in cages with normal diet and water present. Six hours post dose, food is removed from the cages. Animal necropsy is performed 24 hours after LNP dosing. Rats are anesthetized under isoflurane for 5 minutes, then maintained under anesthesia by placing them in nose cones continuing the delivery of isoflurane until ex-sanguination is completed. Blood is collected from the vena cava using a 23 gauge butterfly venipuncture set and aliquoted to serum separator vacutainers for serum chemistry analysis. Punches of the excised caudate liver lobe is taken and placed in RNALater (Ambion) for mRNA analysis. Preserved liver tissue is homogenized and total RNA isolated using a Qiagen bead mill and the Qiagen miRNA-Easy RNA isolation kit following the manufacturer's instructions. Liver ApoB mRNA levels are determined by quantitative RT-PCR. Message is amplified from purified RNA utilizing a rat ApoB commercial probe set (Applied Biosystems Cat # RN01499054_m1). The PCR reaction is performed on an ABI 7500 instrument with a 96-well Fast Block. The ApoB mRNA level is normalized to the housekeeping PPIB (NM 011149) mRNA. PPIB mRNA levels are

determined by RT-PCR using a commercial probe set (Applied Biosytems Cat. No. Mm00478295_m1). Results are expressed as a ratio of ApoB mRNA/ PPIB mRNA. All mRNA data is expressed relative to the PBS control dose. Serum ALT and AST analysis is performed on the Siemens Advia 1800 Clinical Chemistry Analyzer utilizing the Siemens alanine aminotransferase (Cat# 03039631) and aspartate aminotransferase (Cat# 03039631) reagents.

EXAMPLE 5

Determination of Cationic Lipid Levels in Rat/Monkey Liver

Liver tissue is weighed into 20-ml vials and homogenized in 9 v/w of water using a GenoGrinder 2000 (OPS Diagnostics, 1600 strokes/min, 5min). A 50 μL aliquot of each tissue homogenate is mixed with 300 μL of extraction/protein precipitating solvent (50/50 acetonitrile/methanol containing 500 nM internal standard) and the plate is centrifuged to sediment precipitated protein. A volume of 200 μL of each supernatant is then transferred to separate wells of a 96-well plate and 10 μl samples were directly analyzed by LC/MS-MS.

Standards are prepared by spiking known amounts of a methanol stock solution of compound into untreated rat liver homogenate (9 vol water/weight liver). Aliquots (50 μL) each standard/liver homogenate is mixed with 300 μL of extraction/protein precipitating solvent (50/50 acetonitrile/methanol containing 500 nM internal standard) and the plate is centrifuged to sediment precipitated protein. A volume of 200 μL of each supernatant is transferred to separate wells of a 96-well plate and 10 μl of each standard is directly analyzed by LC/MS-MS.

Absolute quantification versus standards prepared and extracted from liver homogenate is performed using an Aria LX-2 HPLC system (Thermo Scientific) coupled to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems). For each run, a total of 10 μL sample is injected onto a BDS Hypersil C8 HPLC column (Thermo, 50 x 2mm, 3 μm) at ambient temperature.

Mobile Phase A: 95% H2O/5% methanol/10 mM ammonium formate/0.1%formic acid *Mobile Phase B:* 40% methanol/60% n-propanol/10 mM ammonium formate/0.1%formic acid The flow rate is 0.5 mL/min and gradient elution profile is as follows: hold at 80% A for 0.25 min, linear ramp to 100% B over 1.6 min, hold at 100% B for 2.5 min, then return and hold at 80% A for 1.75 min. Total run time is 5.8 min. API 4000 source parameters is CAD: 4, CUR: 15, GS1: 65, GS2: 35, IS: 4000, TEM: 550, CXP: 15, DP: 60, EP: 10.

EXAMPLE 6

Rhesus Monkey *In Vivo* Evaluation of ApoB Efficacy

LNPs utilizing compounds in the nominal compositions described above, are evaluated for *in vivo* efficacy in male or female Macaca mulatta (rhesus) monkeys. The siRNA targets the mRNA transcript for the ApoB gene (Accession # XM 001097404). The primary sequence and chemical modification pattern of the ApoB siRNA is displayed above. The RDVs (containing siRNA) in PBS vehicle are administered by intravenous injection in the saphenous vein at an injection rate of 20 mL/minute to a dose level of 0.25 mg/kilogram siRNA. The injection volumes are from 1.9 to 2.1 mL/kilogram and monkeys can range in weight from 2.5 to 4.5 kilograms. The RDV or PBS control is administered to three monkeys. At multiple days post dose, 1 mL blood samples are drawn from the femoral artery for serum chemistry analysis. Monkeys are fasted overnight prior to blood draws. As a measure of efficacy, LDL-C is monitored as a downstream surrogate marker of ApoB mRNA reduction.

EXAMPLE 7

Rhesus Monkey *In Vivo* Evaluation of β-catenin Efficacy

On study day -7 predose liver biopsy samples (~0.5-1 gram/sample) are collected from male rhesus monkeys by laparoscopic surgical resection (resection of one biopsy sample from outer edge of one randomly selected liver lobe per monkey). A 5 mm tissue punch is used to sample three non-adjacent ~50 mg samples from each predose biopsy. Samples are preserved in RNAlater™ (Ambion) for later CTNNB1 mRNA analysis.

On study day 0 monkeys are administered suspensions of the lipid nanoparticle (LNP) test articles in phosphate buffered saline (0.05-0.1 mg siRNA/mL) via single-dose intravenous bolus injection at target doses of 0.67, 1.34 or 3.34 mg siRNA/m^2. For dosing purposes, body surface area (m^2) is estimated from body weight according to the established allometric scaling relationship given below (1):

$$BSA\ (m^2) = 0.11 * BW(in\ kg)^{0.65}$$

On study days 2 and 7, at 48 hours and 168 hrs post LNP administration, liver biopsy samples (~0.5-1 gram/sample) are collected from monkeys by laparoscopic surgical resection (2 separate randomly selected liver lobes were resected per monkey). A 5 mm tissue

punch is used to sample three non-adjacent ~50 mg samples per each 48 hr and 168 hr surgical biopsy sample. Samples are preserved in RNAlater™ (Ambion) for later CTNNB1 mRNA analysis.

CTNNB1 mRNA levels are measured by relative quantitative RT-PCR using a primer/probe set validated for CTNNB1 and normalized against mRNA levels of peptidylprolyl isomerase B (also known as PPIB or cyclophilin B) and RNA levels of 18S ribosomal RNA (18S rRNA) . Change in CTNNB1 mRNA liver expression are measured as the difference in PCR threshold cycle number (ΔΔCt) between post-dose samples and each corresponding monkey's predose liver samples.

Calculation of CTNNB1 mRNA knockdown (with respect to pretreatment levels) is calculated from ΔΔCt using the following relationship:

mRNA (% knockdown)= 100- $(100/2^{-\Delta\Delta Ct})$

(1) **FDA Guidance Document**: "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" July 2005, US Department of Health and Human Services, Food and Drug Administration- Center for Drug Evaluation and Research (CDER)

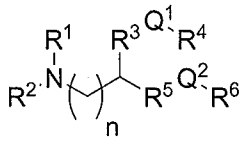
EXAMPLE 8

Rhesus Monkey *In Vivo* Evaluation of ALT Increases

Alanine aminotransferase (ALT) is measured in serum that is harvested from clotted monkey whole blood after centrifugation. A Roche Modular System automated chemistry analyzer measures the enzymatic activity of ALT in the serum by using International Federation of Clinical Chemistry standardized procedures and reagents. The analyzer's computer uses absorbance measurements to calculated ALT activity in the sample as compared to a standard curve. The ALT activity is reported in International Units per Liter (IU/L).

WHAT IS CLAIMED IS:

1. A cationic lipid of Formula A:



A

wherein:

R^1 and R^2 are independently selected from H, $(C_1\text{-}C_6)$alkyl, heterocyclyl, and polyamine, wherein said alkyl, heterocyclyl and polyamine are optionally substituted with one to three substituents selected from R', or R^1 and R^2 can be taken together with the nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocyclic heterocycle is optionally substituted with one to three substituents selected from R';

R^3 is independently selected from $(C_4\text{-}C_{20})$alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from $(C_1\text{-}C_{16})$alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from $(C_4\text{-}C_8)$ alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from $(C_1\text{-}C_2)$alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond, -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -S-S-, -C(R")=N-, -N=C(R")-, -C(R")=N-O-, -O-N=C(R")-, -C(O)(NR")-, -N(R")C(O)-, C(S)(NR")-, -N(R")C(O)-, -N(R")C(O)N(R")-, -OC(O)O-, $OSi(R")_2O$-, $-C(O)(CR"_2)C(O)O$-, or –

$OC(O)(CR''_2)C(O)$-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

R' is independently selected from halogen, R", OR", SR", CN, CO_2R'' or $CON(R'')_2$;

R" is independently selected from H and $(C_1\text{-}C_6)$alkyl, wherein said alkyl is optionally substituted with halogen and OH;

n is 0, 1, 2, 3, 4 or 5;

or any pharmaceutically acceptable salt or stereoisomer thereof.

2. A cationic lipid of Formula A according to Claim 1, wherein:

R^1 and R^2 are each methyl;

n is 0;

R^3 is independently selected from $(C_4\text{-}C_{20})$alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from $(C_1\text{-}C_{16})$alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from $(C_4\text{-}C_8)$ alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from $(C_1\text{-}C_2)$alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond or –C(O)O-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

or any pharmaceutically acceptable salt or stereoisomer thereof.

3. A cationic lipid of Formula A according to Claim 1, wherein:

R^1 and R^2 are each methyl;

n is 2;

R^3 is independently selected from (C_4-C_{20})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^4 is independently selected from (C_1-C_{16})alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^5 is independently selected from (C_4-C_8) alkyl or alkenyl, said alkyl or alkenyl optionally substituted with one to three substituents selected from R';

R^6 is independently selected from (C_1-C_2)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

Q^1 and Q^2 are each, independently, a bond or –C(O)O-, with the proviso that when either Q^1 or Q^2 is a bond then the other is not a bond;

or any pharmaceutically acceptable salt or stereoisomer thereof.

4. A cationic lipid which is selected from:

methyl (9Z)-19-(dimethylamino)octacos-9-enoate (Compound 1);

methyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 2);
methyl (9Z)-19-(dimethylamino)heptacos-9-enoate (Compound 3);
methyl (9Z)-19-(dimethylamino)hexacos-9-enoate (Compound 4);
methyl (9Z)-19-(dimethylamino)pentacos-9-enoate (Compound 5);
methyl (9Z)-21-(dimethylamino)triacont-9-enoate (Compound 6);
methyl (9Z)-21-(dimethylamino)nonacos-9-enoate (Compound 7);
methyl (9Z)-21-(dimethylamino)octacos-9-enoate (Compound 8);
methyl (9Z)-21-(dimethylamino)heptacos-9-enoate (Compound 9);
methyl (11Z)-19-(dimethylamino)octacos-11-enoate (Compound 10);
methyl (7Z)-19-(dimethylamino)octacos-7-enoate (Compound 11);
methyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 12);
methyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 13);
methyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate (Compound 14);
methyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate (Compound 15);
methyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate (Compound 16);
methyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 17);
methyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 18);
methyl 10-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}decanoate (Compound 19);
methyl 6-{2-[11-(dimethylamino)icosyl]cyclopropyl}hexanoate (Compound 20);
ethyl (7Z)-17-(dimethylamino)hexacos-7-enoate (Compound 21);
ethyl (7Z)-17-(dimethylamino)pentacos-7-enoate (Compound 22);
ethyl (7Z)-17-(dimethylamino)tetracos-7-enoate (Compound 23);
ethyl (7Z)-17-(dimethylamino)tricos-7-enoate (Compound 24);
ethyl (9Z)-17-(dimethylamino)hexacos-9-enoate (Compound 25);
ethyl (5Z)-17-(dimethylamino)hexacos-5-enoate (Compound 26);
ethyl (9Z)-19-(dimethylamino)octacos-9-enoate (Compound 27);
ethyl (9Z)-19-(dimethylamino)heptacos-9-enoate (Compound 28);
ethyl (9Z)-19-(dimethylamino)hexacos-9-enoate (Compound 29);
ethyl (9Z)-19-(dimethylamino)pentacos-9-enoate (Compound 30);
ethyl (9Z)-21-(dimethylamino)triacont-9-enoate (Compound 31);
ethyl (9Z)-21-(dimethylamino)nonacos-9-enoate (Compound 32);
ethyl (9Z)-21-(dimethylamino)octacos-9-enoate (Compound 33);
ethyl (9Z)-21-(dimethylamino)heptacos-9-enoate (Compound 34);

ethyl 6-{2-[9-(dimethylamino)octadecyl]cyclopropyl}hexanoate (Compound 35);
ethyl 6-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}hexanoate (Compound 36);
ethyl 6-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}hexanoate (Compound 37);
ethyl 6-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}hexanoate (Compound 38);
ethyl 8-{2-[7-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 39);
ethyl 4-{2-[11-(dimethylamino)icosyl]cyclopropyl}butanoate (Compound 40);
ethyl 8-{2-[9-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 41);
ethyl 8-{2-[9-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 42);
ethyl 8-{2-[9-(dimethylamino)hexadecyl]cyclopropyl}octanoate (Compound 43);
ethyl 8-{2-[9-(dimethylamino)pentadecyl]cyclopropyl}octanoate (Compound 44);
ethyl 8-{2-[11-(dimethylamino)icosyl]cyclopropyl}octanoate (Compound 45);
ethyl 8-{2-[11-(dimethylamino)nonadecyl]cyclopropyl}octanoate (Compound 46);
ethyl 8-{2-[11-(dimethylamino)octadecyl]cyclopropyl}octanoate (Compound 47);
ethyl 8-{2-[11-(dimethylamino)heptadecyl]cyclopropyl}octanoate (Compound 48);
methyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate (Compound 49);
methyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 50);
methyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate (Compound 51);
methyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate (Compound 52);
methyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate (Compound 53);
methyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate (Compound 54);
methyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate (Compound 55);
methyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate (Compound 56);
methyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate (Compound 57);
methyl (11Z)-19-[(dimethylamino)methyl]octacos-11-enoate (Compound 58);
methyl (7Z)-19-[(dimethylamino)methyl]octacos-7-enoate (Compound 59);
methyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 60);
methyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 61);
methyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate (Compound 62);
methyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate (Compound 63);
methyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate (Compound 64);
methyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 65);
methyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 66);
methyl 10-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)decanoate (Compound 67);

methyl 6-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)hexanoate (Compound 68);
ethyl (7Z)-17-[(dimethylamino)methyl]hexacos-7-enoate (Compound 69);
ethyl (7Z)-17-[(dimethylamino)methyl]pentacos-7-enoate (Compound 70);
ethyl (7Z)-17-[(dimethylamino)methyl]tetracos-7-enoate (Compound 71);
ethyl (7Z)-17-[(dimethylamino)methyl]tricos-7-enoate (Compound 72);
ethyl (9Z)-17-[(dimethylamino)methyl]hexacos-9-enoate (Compound 73);
ethyl (5Z)-17-[(dimethylamino)methyl]hexacos-5-enoate (Compound 74);
ethyl (9Z)-19-[(dimethylamino)methyl]octacos-9-enoate (Compound 75);
ethyl (9Z)-19-[(dimethylamino)methyl]heptacos-9-enoate (Compound 76);
ethyl (9Z)-19-[(dimethylamino)methyl]hexacos-9-enoate (Compound 77);
ethyl (9Z)-19-[(dimethylamino)methyl]pentacos-9-enoate (Compound 78);
ethyl (9Z)-21-[(dimethylamino)methyl]triacont-9-enoate (Compound 79);
ethyl (9Z)-21-[(dimethylamino)methyl]nonacos-9-enoate (Compound 80);
ethyl (9Z)-21-[(dimethylamino)methyl]octacos-9-enoate (Compound 81);
ethyl (9Z)-21-[(dimethylamino)methyl]heptacos-9-enoate (Compound 82);
ethyl 6-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)hexanoate (Compound 83);
ethyl 6-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)hexanoate (Compound 84);
ethyl 6-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)hexanoate (Compound 85);
ethyl 6-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)hexanoate (Compound 86);
ethyl 8-(2-{7-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 87);
ethyl 4-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)butanoate (Compound 88);
ethyl 8-(2-{9-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 89);
ethyl 8-(2-{9-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 90);
ethyl 8-(2-{9-[(dimethylamino)methyl]hexadecyl}cyclopropyl)octanoate (Compound 91);
ethyl 8-(2-{9-[(dimethylamino)methyl]pentadecyl}cyclopropyl)octanoate (Compound 92);
ethyl 8-(2-{11-[(dimethylamino)methyl]icosyl}cyclopropyl)octanoate (Compound 93);
ethyl 8-(2-{11-[(dimethylamino)methyl]nonadecyl}cyclopropyl)octanoate (Compound 94);
ethyl 8-(2-{11-[(dimethylamino)methyl]octadecyl}cyclopropyl)octanoate (Compound 95);
ethyl 8-(2-{11-[(dimethylamino)methyl]heptadecyl}cyclopropyl)octanoate (Compound 96);
methyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 97);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 98);
methyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 99);
methyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 100);

methyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate (Compound 101);
methyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate (Compound 102);
methyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate (Compound 103);
methyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 104);
methyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 105);
methyl (11Z)-19-[2-(dimethylamino)ethyl]octacos-11-enoate (Compound 106);
methyl (7Z)-19-[2-(dimethylamino)ethyl]octacos-7-enoate (Compound 107);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 108);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 109);
methyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate (Compound 110);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate (Compound 111);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate (Compound 112);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 113);
methyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 114);
methyl 10-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)decanoate (Compound 115);
methyl 6-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)hexanoate (Compound 116);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]hexacos-7-enoate (Compound 117);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]pentacos-7-enoate (Compound 118);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]tetracos-7-enoate (Compound 119);
ethyl (7Z)-17-[2-(dimethylamino)ethyl]tricos-7-enoate (Compound 120);
ethyl (9Z)-17-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 121);
ethyl (5Z)-17-[2-(dimethylamino)ethyl]hexacos-5-enoate (Compound 122);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 123);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 124);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]hexacos-9-enoate (Compound 125);
ethyl (9Z)-19-[2-(dimethylamino)ethyl]pentacos-9-enoate (Compound 126);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]triacont-9-enoate (Compound 127);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]nonacos-9-enoate (Compound 128);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]octacos-9-enoate (Compound 129);
ethyl (9Z)-21-[2-(dimethylamino)ethyl]heptacos-9-enoate (Compound 130);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)hexanoate (Compound 131);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)hexanoate (Compound 132);
ethyl 6-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)hexanoate (Compound 133);

ethyl 6-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)hexanoate (Compound 134);
ethyl 8-(2-{7-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 135);
ethyl 4-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)butanoate (Compound 136);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 137);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 138);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]hexadecyl}cyclopropyl)octanoate (Compound 139);
ethyl 8-(2-{9-[2-(dimethylamino)ethyl]pentadecyl}cyclopropyl)octanoate (Compound 140);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]icosyl}cyclopropyl)octanoate (Compound 141);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]nonadecyl}cyclopropyl)octanoate (Compound 142);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]octadecyl}cyclopropyl)octanoate (Compound 143);
ethyl 8-(2-{11-[2-(dimethylamino)ethyl]heptadecyl}cyclopropyl)octanoate (Compound 144);
(2Z)-non-2-en-1-yl 10-(dimethylamino)nonadecanoate (Compound 145);
(2-hexylcyclopropyl)methyl 10-(dimethylamino)nonadecanoate (Compound 146);
(2Z)-undec-2-en-1-yl 8-(dimethylamino)heptadecanoate (Compound 147);
(2Z)-hept-2-en-1-yl 12-(dimethylamino)henicosanoate (Compound 148);
(2-octylcyclopropyl)methyl 8-(dimethylamino)heptadecanoate (Compound 149);
(2-butylcyclopropyl)methyl 12-(dimethylamino)henicosanoate (Compound 150);
(2Z)-non-2-en-1-yl 10-[(dimethylamino)methyl]nonadecanoate (Compound 151);
(2Z)-undec-2-en-1-yl 8-[(dimethylamino)methyl]heptadecanoate (Compound 152);
(2Z)-hept-2-en-1-yl 12-[(dimethylamino)methyl]henicosanoate (Compound 153);
(2-hexylcyclopropyl)methyl 10-[(dimethylamino)methyl]nonadecanoate (Compound 154);
(2-octylcyclopropyl)methyl 8-[(dimethylamino)methyl]heptadecanoate (Compound 155);
(2-butylcyclopropyl)methyl 12-[(dimethylamino)methyl]henicosanoate (Compound 156);
(2Z)-non-2-en-1-yl 10-[2-(dimethylamino)ethyl]nonadecanoate (Compound 157);
(2Z)-undec-2-en-1-yl 8-[2-(dimethylamino)ethyl]heptadecanoate (Compound 158);
(2Z)-hept-2-en-1-yl 12-[2-(dimethylamino)ethyl]henicosanoate (Compound 159);
(2-hexylcyclopropyl)methyl 10-[2-(dimethylamino)ethyl]nonadecanoate (Compound 160);
(2-octylcyclopropyl)methyl 8-[2-(dimethylamino)ethyl]heptadecanoate (Compound 161);
(2-butylcyclopropyl)methyl 12-[2-(dimethylamino)ethyl]henicosanoate (Compound 162);
methyl (19Z,22Z)-9-(dimethylamino)octacosa-19,22-dienoate (Compound 163);
ethyl (18Z,21Z)-8-(dimethylamino)heptacosa-18,21-dienoate (Compound 164);
methyl 9-(dimethylamino)-16-(2-octylcyclopropyl)hexadecanoate (Compound 165);
ethyl 8-(dimethylamino)-15-(2-octylcyclopropyl)pentadecanoate (Compound 166);

methyl (19Z,22Z)-9-[(dimethylamino)methyl]octacosa-19,22-dienoate (Compound 167);
ethyl (18Z,21Z)-8-[(dimethylamino)methyl]heptacosa-18,21-dienoate (Compound 168);
methyl 9-[(dimethylamino)methyl]-16-(2-octylcyclopropyl)hexadecanoate (Compound 169);
ethyl 8-[(dimethylamino)methyl]-15-(2-octylcyclopropyl)pentadecanoate (Compound 170);
methyl (19Z,22Z)-9-[2-(dimethylamino)ethyl]octacosa-19,22-dienoate (Compound 171);
ethyl (18Z,21Z)-8-[2-(dimethylamino)ethyl]heptacosa-18,21-dienoate (Compound 172);
methyl 9-[2-(dimethylamino)ethyl]-16-(2-octylcyclopropyl)hexadecanoate (Compound 173);
ethyl 8-[2-(dimethylamino)ethyl]-15-(2-octylcyclopropyl)pentadecanoate (Compound 174);
dimethyl (9Z)-19-(dimethylamino)heptacos-9-enedioate (Compound 175); and
1-methyl 18-[(2Z)-non-2-en-1-yl] 9-(dimethylamino)octadecanedioate (Compound 176);

or any pharmaceutically acceptable salt or stereoisomer thereof.

5. An LNP composition which comprises, a cationic lipid of Formula A according to Claim 1, cholesterol, DSPC and PEG-DMG.

6. The use of a cationic lipid according to Claim 1 for the preparation of lipid nanoparticles.

7. The use of a cationic lipid according to Claim 1 as a component in a lipid nanoparticle for the delivery of oligonucleotides.

8. The use according to Claim 9 wherein the oligonucleotides are siRNA.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/23359

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 45/00; C08H 3/00, C11D 1/28; A01N 25/00 (2013.01)
USPC - 424/283.1
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)
USPC: 424/283.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 554/110, 514/785, 514/44A, 514/44R (keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents/Scholar
Search Terms Used: Cationic lipid, ester bond, biodegradable, acyl ester, reversed orientation, asymmetric, bipolar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/153493 A2 (Manoharan, et al.) 08 December 2011 (08.12.2011) pg 4, para 3 to pg 5, para 1, pg 13, para 6, pg 27, para 3, pg 97, para 2-3, Tables 1-2, Claims 25-26	1-2, 4-8
Y	Wetzer, et al. Reducible cationic lipids for gene transfer. Biochem J 2001, 356:747-756; abstract, pg 748, Fig. 1, pg 754, col 1, para 3, col 2, para 1	1-2, 4-8
A	WO 2003/094971 A1 (Leong, et al.) 20 November 2003 (20.11.2003) whole doc.	1-2, 4-8
A	Montier, et al. Progress in Cationic Lipid-Mediated Gene Transfection: A Series of Bio-Inspired Lipids as an Example. Current Gene Therapy 2008, 8:296-312; Fig 13, compound 17	1-2, 4-8
A	PubChem compound CID 599141. 2005. [Retrieved from the Internet 23 March 2013: <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=599141>] whole doc.	1

☐ Further documents are listed in the continuation of Box C. ☐

* 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
06 June 2013 (04.06.2013)	19 JUN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/23359

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Group I+: claims 1-8, drawn to a cationic lipid of Formula A. The first invention (Claims 1-2, 4-8) is restricted to compound 1 of claim 4 and will be searched without additional fee. Applicant is invited to elect additional compounds, by paying additional fee per each compound or invention. An exemplary election is compound 2 of claim 4 (Claims 1-2, 4-8). The exact claims searched will depend on Applicant's election. Failure to clearly identify how any paid additional invention fees are to be applied to the Group I+ will result in only the first claimed invention to be searched.

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Groups I+ is a specific compound.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2, 4-8, restricted to compound 1 of claim 4

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/23359

Box NO III. Observations where unity of invention is lacking

Common Technical Features
The inventions of Group I+ share the technical feature of a cationic lipid of Formula A. However, this shared technical feature does not represent a contribution over prior art as being anticipated by PubChem compound CID 599141 (hereinafter "CID 599141") that teaches a compound of Formula A wherein R1 and R2 are independently methyl, R3 is C10-alkyl, R4 is C2-alkyl, R5 is C4-alkyl, R6 is C1-alkyl, Q1 is a bond, Q2 is -OC(O)-, n is 0 (pg 1, methyl 6-(dimethylamino)octadecanoate). It should be noted that methyl 6-(dimethylamino)octadecanoate of CID 599141 is a free form cationic lipid like methyl (9Z)-19-(dimethyl amino)octacos-9-enoate (Compound 1, claim 4). Further, it should also be noted that if a free form compound such as methyl (9Z)-19-(dimethyl amino)octacos-9-enoate (Compound 1, claim 4) is considered a cationic lipid, by the same token, methyl 6-(dimethylamino)octadecanoate methyl 6-(dimethylamino)octadecanoate of CID 599141 must be a cationic lipid as well. In addition, it should be noted that the free forms such as methyl 6-(dimethylamino)octadecanoate methyl 6-(dimethylamino)octadecanoate of CID 599141 or methyl (9Z)-19-(dimethyl amino)octacos-9-enoate (Compound 1, claim 4) may differ from their respective salt forms somewhat in certain physical properties, such as solubility in polar solvents, but the acid and base salts are otherwise pharmaceutically equivalent to their respective free forms for purposes of the invention (Applicant's Specification, pg 15, ln 12-15).

As the common technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.