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(54) **LOW MOLECULAR WEIGHT CATIONIC LIPIDS FOR OLIGONUCLEOTIDE DELIVERY**

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Related U.S. Application Data

(63) Continuation of application No. 14/681,641, filed on Apr. 8, 2015, now Pat. No. 9,458,087, which is a continuation of application No. 13/876,528, filed as application No. PCT/US2011/053556 on Sep. 28, 2011, now Pat. No. 9,029,604.

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(51) **Int. Cl.**

C12N 15/113	(2010.01)
C07C 217/08	(2006.01)
C12N 15/88	(2006.01)
A61K 9/51	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/113*** (2013.01); ***A61K 9/5123*** (2013.01); ***C07C 217/08*** (2013.01); ***C12N 15/88*** (2013.01); *C07C 2101/02* (2013.01); *C12N 2310/14* (2013.01); *C12N 2320/32* (2013.01); *Y10S 977/783* (2013.01); *Y10S 977/916* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(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 to enhance the efficiency and tolerability of in vivo delivery of siRNA.

15 Claims, 2 Drawing Sheets

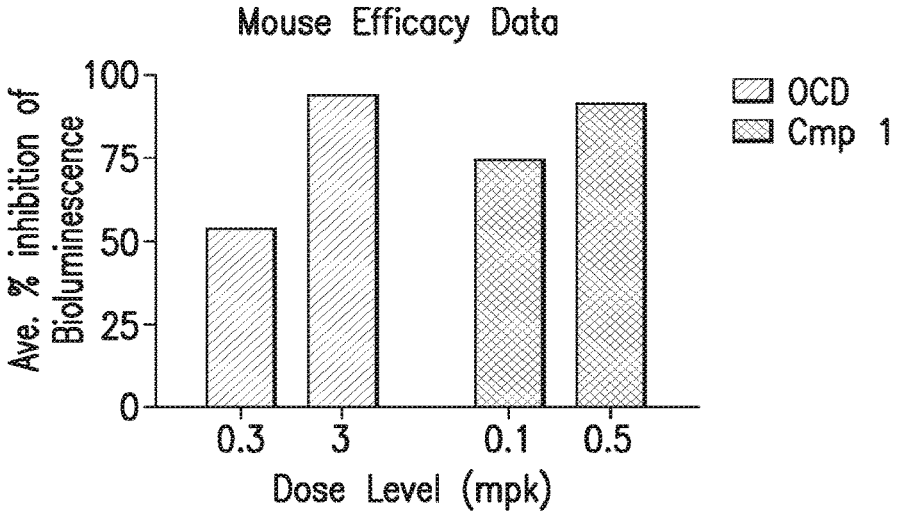

Mouse Efficacy Data
Ave. % inhibition of Bioluminescence
100
75
50
25
0
0.3
3
0.1
0.5
Dose Level (mpk)
OCD
Cmp 1

FIG. 1

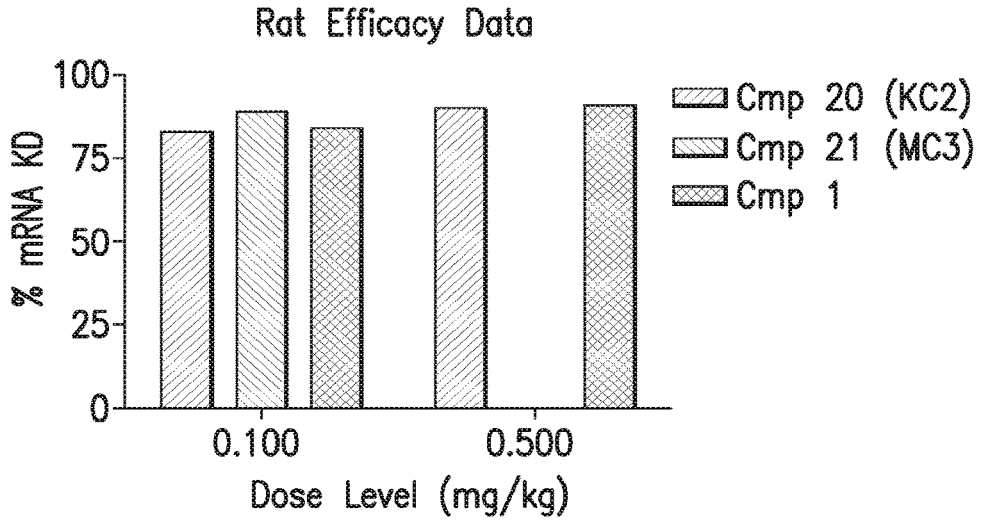

Rat Efficacy Data
% mRNA KD
100
75
50
25
0
0.100
0.500
Dose Level (mg/kg)
Cmp 20 (KC2)
Cmp 21 (MC3)
Cmp 1

FIG. 2

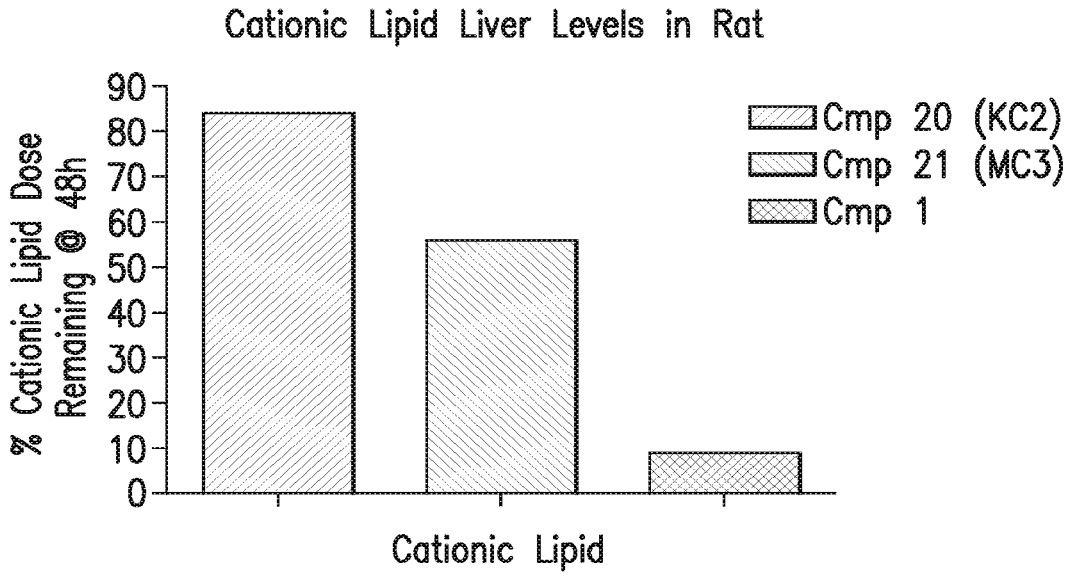

Cationic Lipid Liver Levels in Rat
% Cationic Lipid Dose Remaining @ 48h
90
80
70
60
50
40
30
20
10
0
Cmp 20 (KC2)
Cmp 21 (MC3)
Cmp 1
Cationic Lipid

FIG.3

LOW MOLECULAR WEIGHT CATIONIC LIPIDS FOR OLIGONUCLEOTIDE DELIVERY

This application is a Continuation of U.S. patent application Ser. No. 14/681,641 filed Apr. 8, 2015, now U.S. Pat. No. 9,458,087 issued Oct. 4, 2016, which is a Continuation of U.S. patent application Ser. No. 13/876,528 filed Mar. 28, 2013, now U.S. Pat. No. 9,029,604 issued May 12, 2015, which is 371 National Phase Entry of International Patent Application No. PCT/US2011/53556 filed Sep. 28, 2011, and which claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/388,201, filed Sep. 30, 2010, the contents of which are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The sequence listing of the present application has been submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "MRLMIS00045WOPCTSEQ.txt", creation date of Sep. 29, 2016 and a size of 4,161 bytes. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

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 U.S. patent applications: U.S. 2006/0083780, U.S. 2006/0240554, U.S. 2008/0020058, U.S. 2009/0263407 and U.S. 2009/0285881 and PCT patent applications: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405 and WO2010/054406). See also Semple S. C. et al., Rational design of cationic lipids for siRNA delivery, Nature Biotechnology, published online 17 Jan. 2010; doi:10.1038/nbt.1602.

Other cationic lipids are disclosed in U.S. patent applications: U.S. 2009/0263407, U.S. 2009/0285881, U.S. 2010/0055168, U.S. 2010/0055169, U.S. 2010/0063135, U.S. 2010/0076055, U.S. 2010/0099738 and U.S. 2010/0104629.

Traditional cationic lipids such as CLinDMA and DLinDMA have been employed for siRNA delivery to 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 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 to enhance the efficiency and tolerability of in vivo delivery of siRNA.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**: LNP (Compound 1) efficacy in mice.

FIG. **2**. LNP (Compound 1) efficacy in rat (ApoB siRNA).

FIG. **3**. Cationic lipid (Compound 1) levels in rat liver.

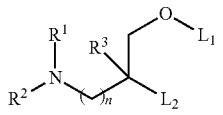
DETAILED DESCRIPTION OF THE INVENTION

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 U.S. patent applications: U.S. 2006/0083780, U.S. 2006/0240554, U.S. 2008/0020058, U.S. 2009/0263407 and U.S. 2009/0285881 and PCI patent applications: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405 and WO2010/054406). See also Semple S. C. et al., Rational design of cationic lipids for siRNA delivery, Nature Biotechnology, published online 17 Jan. 2010; doi:10.1038/nbt.1602.

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-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 selected from H and (C_1-C_6)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

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

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; and

L_1 and L_2 are independently selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl, said alkyl and alkenyl are optionally substituted with one or more substituents selected from R';

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:

R^3 is H;

b is 0;

L_1 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl; and

L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl;

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;

R^3 is H;

n is 0;

L_1 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl; and

L_2 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl;

or any pharmaceutically acceptable salt or stereoisomer thereof.

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

R^1 and R^2 are each methyl;

R^3 is H;

n is 1;

L_1 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl; and

L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl;

or any pharmaceutically acceptable salt or stereoisomer thereof.

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

R^1 and R^2 are each methyl;

R^3 is H;

n is 2;

L_1 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl; and

L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl;

or any pharmaceutically acceptable salt, or stereoisomer thereof.

Specific cationic lipids are:

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (Compound 1);

(2S)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (Compound 2);

(2S)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]dodecan-2-amine (Compound 3);

(2R)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]dodecan-2-amine (Compound 4);

(2S)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]decan-2-amine (Compound 5);

(2S)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]nonan-2-amine (Compound 6);

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]tridecan-2-amine (Compound 7);

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]nonan-2-amine (Compound 8);

(2R)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]dodecan-2-amine (Compound 9);

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]dodecan-2-amine (Compound 10);

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]decan-2-amine (Compound 11); and

(2S,12Z,15Z)—N,N-dimethyl-1-(octyloxy)henicosa-12,15-dien-2-amine (Compound 12);

(2R,12Z,15Z)-1-(decyloxy)-N,N-dimethylhenicosa-12,15-dien-2-amine (Compound 13);

(2R,12Z,15Z)-1-(hexyloxy)-N,N-dimethylhenicosa-12,15-dien-2-amine (Compound 14);

(2R,12Z,15Z)-1-(hexadecyloxy)-N,N-dimethylhenicosa-12,15-dien-2-amine (Compound 15);

(2R,12Z,15Z)—N,N-dimethyl-1-(undecyloxy)henicosa-12,15-dien-2-amine (Compound 16);

N,N-dimethyl-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}undecan-1-amine (Compound 17);

N,N-dimethyl-3-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}dodecan-1-amine (Compound 18); and

(2S)—N,N-dimethyl-1-({8-[(1R,2R)-2-{[(1S,2S)-2-pentylcyclopropyl]methyl}cyclopropyl]octyl}oxy)tridecan-2-amine (Compound 19);

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: EX. 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.

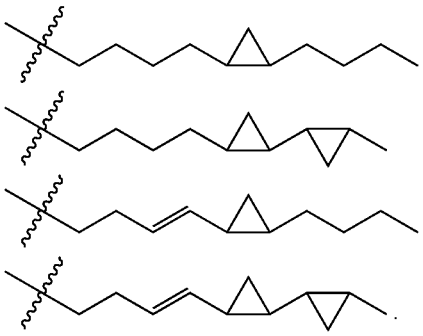
In the compounds of Formula A, the atoms may exhibit their natural isotopic abundances, or one or more of the atoms may be artificially enriched in a particular isotope having the same atomic number, but an atomic mass or mass number different, from the atomic mass or mass number predominantly found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



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: benzoiimdazolyl, 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, dihydroiimdazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydroietrazolyl, dihydrothiadiazolyl, dihydrothiaxolyl, 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-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 selected from H and methyl.

In an embodiment of Formula A, R^3 is H.

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 or 2.

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

In an embodiment of Formula A, n is 0.

In an embodiment of Formula A, L_1 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl, which are optionally substituted with halogen and OH.

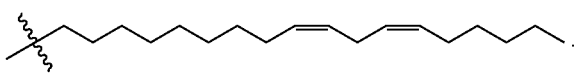
In an embodiment of Formula A, L_1 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl.

In an embodiment of Formula A, L_1 is selected from C_3-C_{24} alkenyl.

In an embodiment of Formula A, L_1 is selected from C_{12}-C_{24} alkenyl.

In an embodiment of Formula A, L_1 is C_{18} alkenyl.

In an embodiment of Formula A, L_1 is:



In an embodiment of Formula A, L_1 is C_8 alkyl.

In an embodiment of Formula A, L_2 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl, which are optionally substituted with halogen and OH.

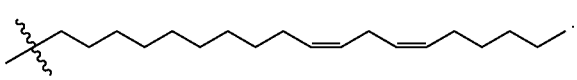
In an embodiment of Formula A, L_2 is selected from C_3-C_{24} alkyl and C_3-C_{24} alkenyl.

In an embodiment of Formula A, L_2 is selected from C_3-C_{24} alkenyl.

In an embodiment of Formula A, L_2 is selected from C_{12}-C_{24} alkenyl.

In an embodiment of Formula A, L_2 is C_{19} alkenyl.

In an embodiment of Formula A, L_2 is:



In an embodiment of Formula A, L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl, which are optionally substituted with halogen and OH.

In an embodiment of Formula A, L_2 is selected from C_5-C_9 alkyl and C_5-C_9 alkenyl, which are optionally substituted with halogen and OH.

In an embodiment of Formula A, L_2 is selected from C_7-C_9 alkyl and C_7-C_9 alkenyl, which are optionally substituted with halogen and OH.

In an embodiment of Formula A, L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl.

In an embodiment of Formula A, L_2 is selected from C_5-C_9 alkyl and C_5-C_9 alkenyl.

In an embodiment of Formula A, L_2 is selected from C_7-C_9 alkyl and C_7-C_9 alkenyl.

In an embodiment of Formula A, L_2 is C_3-C_9 alkyl.

In an embodiment of Formula A, L_2 is C_5-C_9 alkyl.

In an embodiment of Formula A, L_2 is C_7-C_9 alkyl.

In an embodiment of Formula A, L_2 is C_9 alkyl.

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 terra "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 axe 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, diethyiamin, 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.

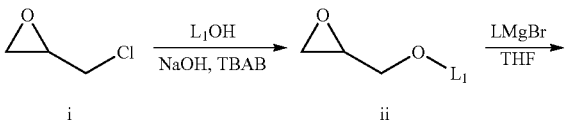
It will also be noted that the cationic lipids of the present invention are potentially internal salts or zwitterions, since under physiological conditions a deprotonated acidic moiety in the compound, such as a carboxyl group, may be anionic, and this electronic charge might then be balanced off Internally 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 are readily prepared by one of ordinary skill in the art.

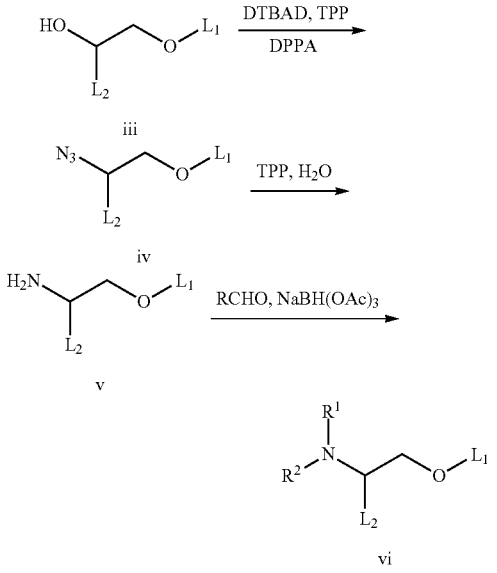
Synthesis of the novel cationic lipids is a linear process starting from epichlorohydrin (i) (General Scheme 1). Epoxide opening, ring closure with lipid alkoxide delivers epoxy ether intermediate ii. Original addition to the epoxide provides secondary alcohol intermediate iii. Mitsinobu inversion with azide followed by reduction yields primary amine intermediates v. Reductive animation provides the tertiary amine derivatives vi.

GENERAL SCHEME 1



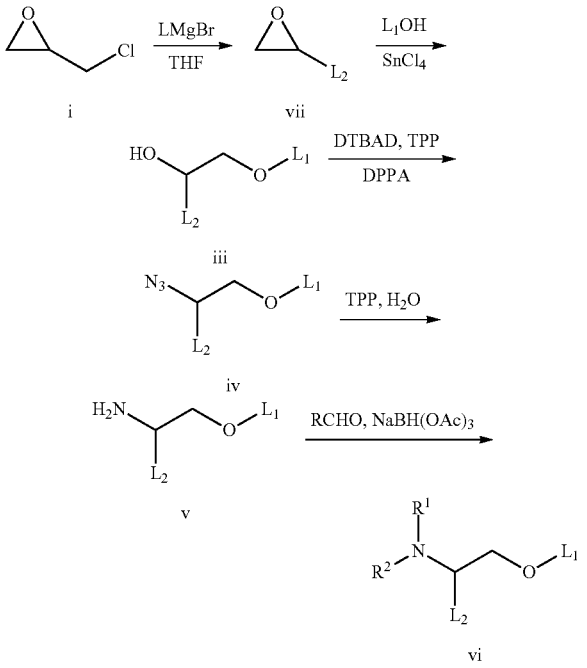
i ii

-continued



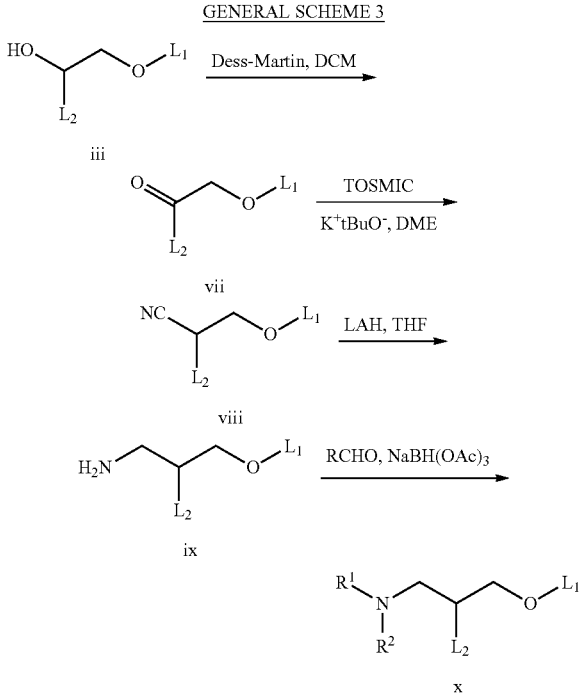
An alternative synthesis of the novel cationic lipids starting from epichlorohydrin (i) is depicted in General Scheme 2. Epoxide opening, ring closure with lipid Grignard delivers epoxide intermediate vii. Alkoxide addition to the epoxide provides secondary alcohol intermediate iii. Mitsinobu inversion with azide followed by reduction yields primary amine intermediates v. Reductive animation provides the tertiary amine derivatives vi.

GENERAL SCHEME 2



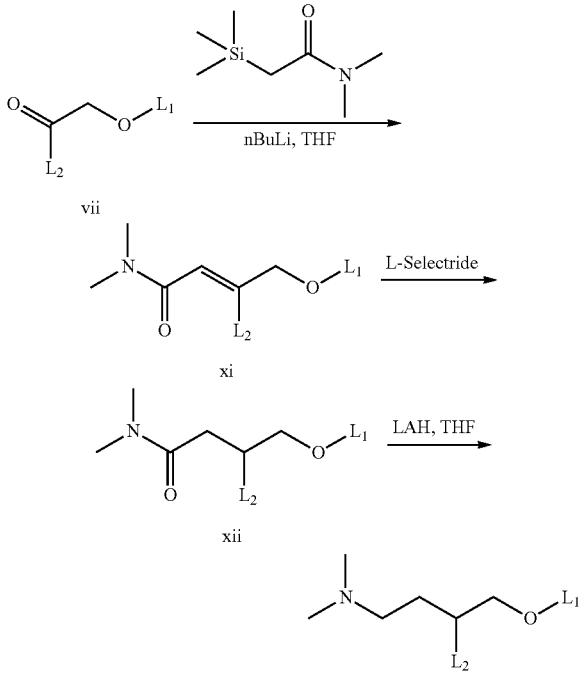
Synthesis of the homologated cationic lipids x (General Scheme 3) begins with oxidation of intermediate iii to ketone vii using Dess-Martin Periodinane. Conversion of the ketone to the nitrile viii is accomplished with TOSMIC. Reduction of the nitrite with lithium aluminum hydride gives primary amine ix. Reductive animation provides cationic lipids x.

GENERAL SCHEME 3

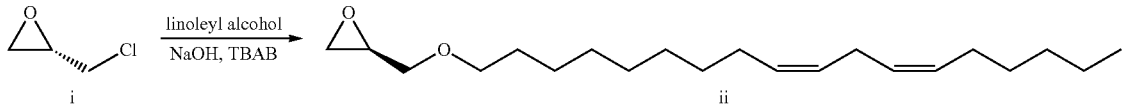


Synthesis of doubly homologated cationic lipids xiii begins with ketone vii. Peterson olefination generates the unsaturated amide xi. Conjugate reduction with L-Selectride gives amide xii. Amide reduction with lithium aluminum hydride gives cationic lipids xiii.

GENERAL SCHEME 4

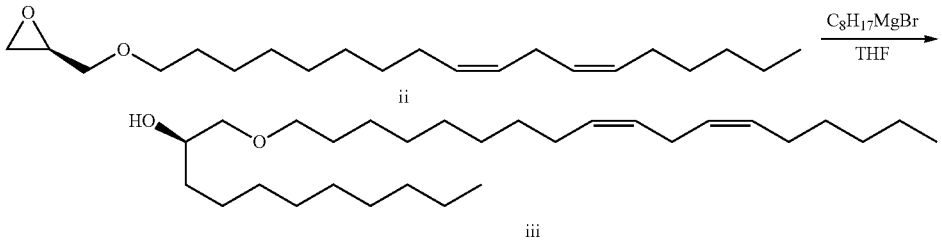


(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (Compound 1)



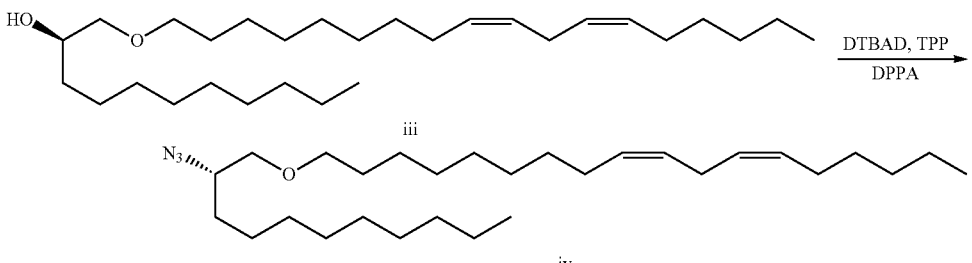
i ii

A 250 ml, rb flask was charged with magnetic stirbar, tetrabutyl ammonium bromide (TBAB, 2.72 g, 8.4 mmol), linoleyl alcohol (225 g, 884 mmol), and sodium hydroxide (50.7 g, 1.2 mol), then cooled in an ice bath. The (S)-epichlorohydrin (156 g, 1.69 mol) was added slowly over 2 hours and then warmed to ambient temperature and stirred overnight, 259 mL of hexane was added and allowed to stir for 15 mins, then mixture was filtered and organic layer was concentrated in vacuo. The product was purified using 0-10% ethyl acetate/hexane gradient on 330 g silica column to give (2R)-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxyl]methyl}oxirane. ^{1}H NMR ($CDCl_3$, 300 mHz) δ 0.90-0.86 (m, 3H), 1.29 (s, 16H), 1.55-1.64 (m, 2H), 2.00-2.07 (m, 4H), 2.58-2.61 (m, 1H), 2.74-2.80 (m, 3H), 3.12-3.15 (m, 1H) 3.34-3.52 (m, 3H), 3.67-3.72 (dd, J=12 Hz, 1H) 5.30-5.35 (m, 4H); HRMS (m+1) calc'd 323.2872. found 323.2951.



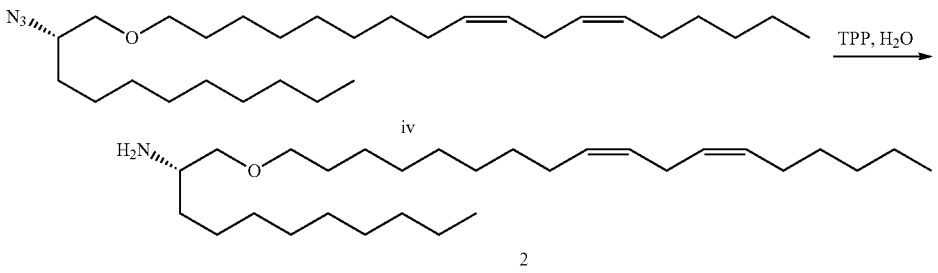
ii iii

The epoxide (15 g, 46.5 mmol) was dissolved in THF and cooled to 0° C. under stream of Nitrogen. Octyl Grignard (25.6 mL 2M solution, 51.2 mmol) was added dropwise and then heated in microwave at 120° C. for one hour. The precipitate was filtered off and the solvent evaporated in vacuo. The crude oil was directly loaded onto a silica gel column and eluted with 0-10% gradient (hexane-ethyl acetate) to give (2R)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-ol. LC/MS (m+1)=437.6.



iii iv

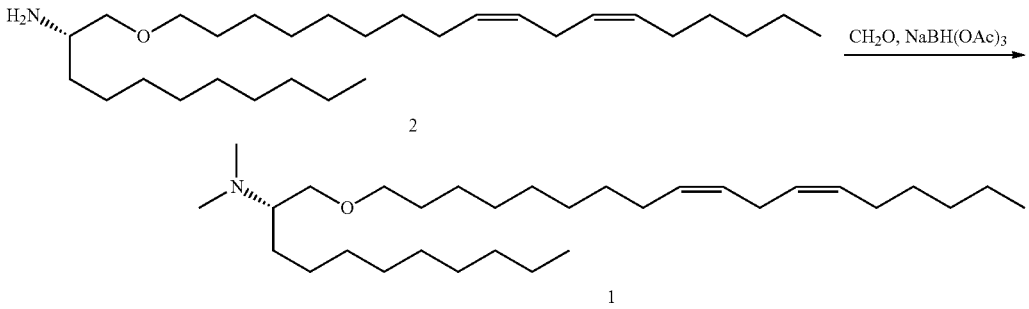
Triphenyl phosphine (14.4 g, 55 mmol) was dissolved in THF and cooled to 0° C. under nitrogen. Di-tertbutyl azodicarboxylate (13.7 g, 59.5 mmol) was added slowly and the reaction was stirred for 30 mins. Then the alcohol (20 g, 45.8 mmol) was added dropwise and allowed to stir for 10 mins, then diphenyl phosphorylazide (15.1 g, 55 mmol) was added and allowed to stir overnight, warming to ambient temperature. The reaction was evaporated to dryness in vacuo and directly loaded onto a silica gel column and eluted with 0-10% ethyl acetate/hexane gradient to provide (2S)-2-azidoundecyl (9Z,12Z)-octadeca-9,12-dien-1-yl ether which was carried directly into the next reaction without characterization.



2

Triphenyl phosphine (4.54 g, 17.3 mmol) and the azide (8 g, 17.3 mmol) were dissolved in THF. The reaction mixture was split into 3 μw tubes and Irradiated at 120° C. for 1 hour each. Considerable pressure built in each tube so care should be noted. LC indicated 100% conversion to phosphoimine intermediate. To each, tube was added ~3 mL of water and the reaction irradiated for 10 min at 120° C. The reaction mixtures were combined and concentrated to remove organic solvent. Hexane was added to precipitate phosphine oxides which were filtered through sintered glass funnel. The solvent was then removed in vacuo. The crude product was purified using HPLC with 30 min run and 60-100% water/acetonitrile gradient. The combined HPLC fractions were neutralized with sodium bicarbonate evaporated in vacuo. The pure product was partitioned between water/hexanes. The organic layer was dried over sodium sulfate, filtered and evaporated in vacuo to afford (2S)-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (2), ^{1}H NMR ($CDCl_3$, 300 mHz) δ 0.88-0.87 (m, 6H), 1.25-1.29 (s, 32H), 1.54-1.54 (m, 2H), 2.03-2.05 (m, 4H), 2.23 (s, 2H), 2.75-2.76 (m, 2H), 2.96 (m, 1H), 3.13-3.18 (m, 1H), 3.38-3.45 (m, 3H), 5.31-5.38 (m, 4H); LC/MS (m+1)=436.7.

by triacetoxy borohydride (5.1 g, 24.1 mmol). The reaction was stirred at ambient temperature for 15 mins. LC/MS indicated 100% conversion to product. Added 1M NaOH until basic and extracted with hexane and washed with water. Retained organic layer and removed solvent in vacuo. Purified using 60-100% water/acetonitrile 30 min gradient on C8 HPLC. Combined fractions and added sodium bicarbonate and evaporated organics in vacuo. The product, was partitioned between water/hexanes and the organics were dried over sodium sulfate, filtered and evaporated in vacuo to deliver (2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (1). ^{1}H NMR ($CDCl_3$, 300 mHz) δ 0.88-0.87 (m, 6H), 1.285 (s, 33H), 1.55 (m, 2H), 1.80 (m, 1H), 2.00-2.05 (s, 4H), 2.29-2.31 (2H), 2.50 (m, 1H), 2.76-1.77 (m, 2H), 3.36-3.51 (m, 6H), 5.34-5.36 (m, 4H); LC/MS (m+1)=464.9.

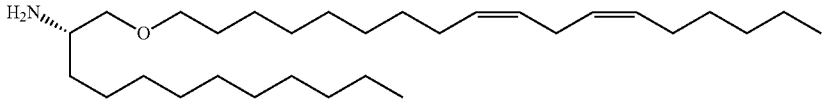
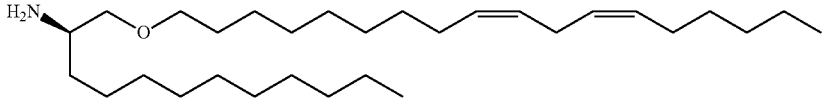


2

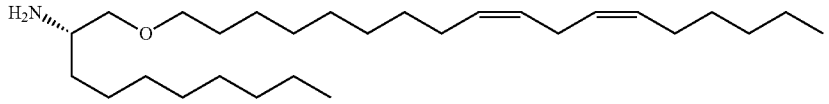
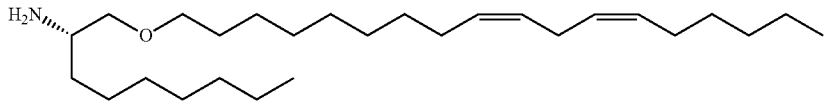
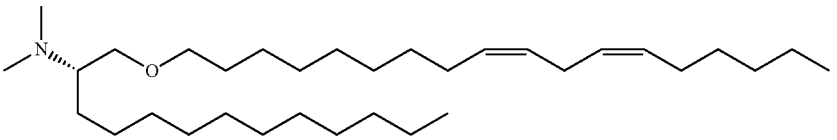
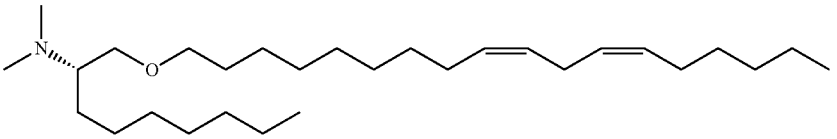
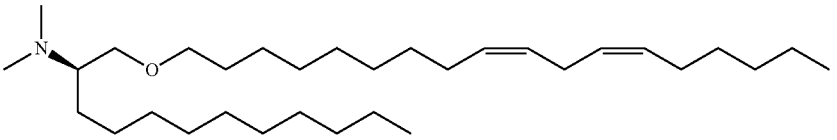
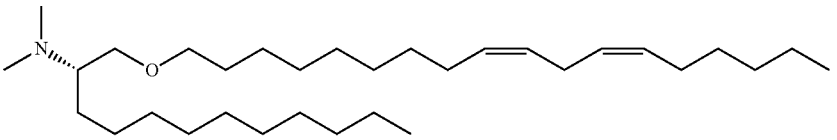
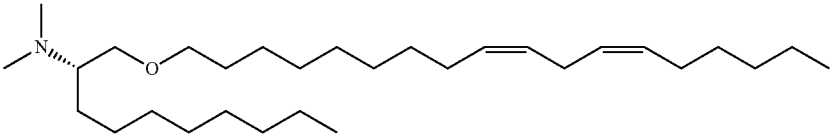
1

The primary amine (3.5 g, 8 mmol) was dissolved in THF and formaldehyde (3.26 g, 40.2 mmol) was added, followed

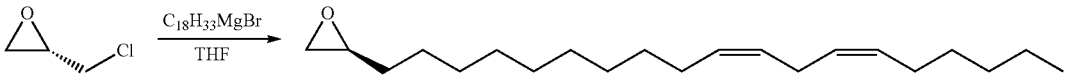
Compounds 3-11 are novel cationic lipids and were prepared according to General Scheme 1 above.

Compound	Structure	LC/MS (m + 1)
3		450.4
4		450.6

-continued

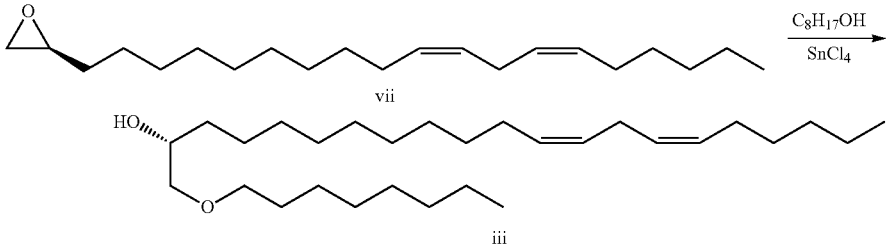
Compound	Structure	LC/MS (m + 1)
5		423.6
6		408.6
7		492.8
8		436.6
9		479.7
10		478.7
11		451.7

(2S)—N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]undecan-2-amine (Compound 12)



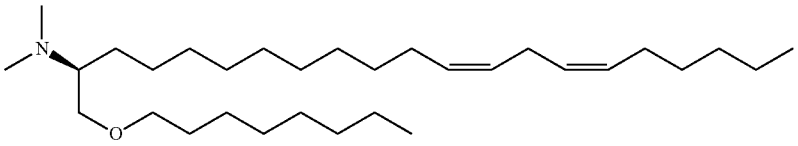
i vii

A round bottomed flask was charged with magnetic stir bar, copper cyanide (1.45 g, 16.2 mmol), epichlorohydrin (15 g, 162 mmol) and purged with nitrogen. THF was added, the solution cooled to −78° C. and linoleyl Grignard (68.8 g, 195 mmol) was added slowly. After addition of Grignard the reaction was allowed to warm to ambient temperature. The reaction was quenched with saturated ammonium chloride solution and extracted with ether. The organics were dried over sodium sulfate, filtered and evaporated in vacuo. The intermediate chloro-alcohol was purified via flash chromatography (silica, 0-35% ethyl acetate/hexanes). The alcohol was dissolved in THF and allowed to stir with solid NaOH pellets at ambient temperature for 16 hours, then filtered off NaOH and washed organic layer with water. The organics were dried over sodium, sulfate, filtered and evaporated in vacuo to provide (2S)-2-[(10Z,13Z)-nonadeca-10,13-dien-1-yl]oxirane. ^{1}H NMR ($CDCl_3$, 300 mHz) δ 0.87-0.90 (m, 3H), 1.27-1.52 (m, 22H), 2.01-2.19 (m, 4H), 2.40-2.46 (m, 1H), 2.71-2.76 (m, 3H), 2.89-2.91 (m, 1H), 5.30-5.36 (m, 4H); LC/MS (m+H+acetonitrile)=349.5.



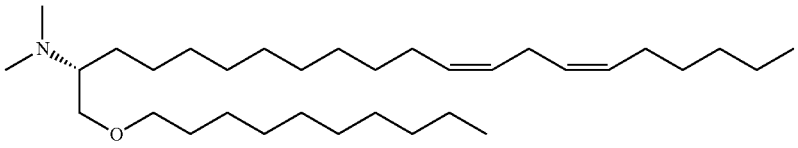
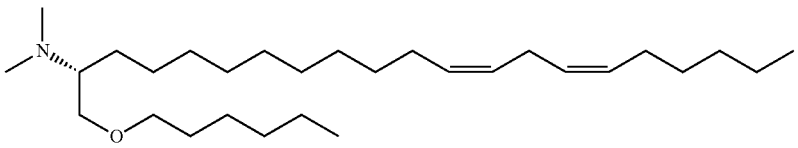
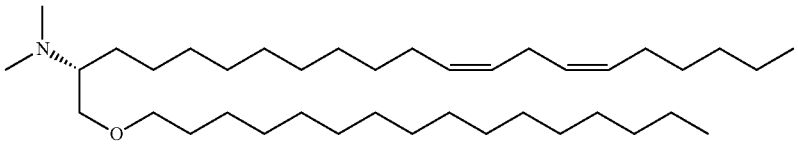
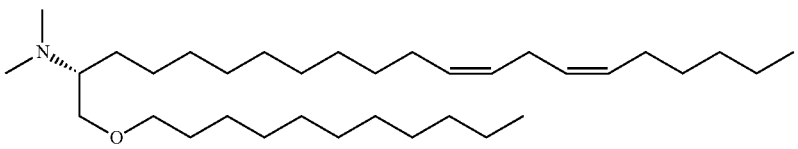
The alcohol (2.55 g, 19.6 mmol) was dissolved in DCM and cooled to 0° C. To this solution was added tin chloride (1.63 mmol, 1.63 mL of a 1M solution). The epoxide (5 g, 16.3 mmol) was added to the reaction mixture dropwise and the reaction was aged for 1 hour at 0° C. The reaction was evaporated in vacuo, dissolved in hexanes and purified by flash chromatography (0-20% ethyl acetate/hexanes) to give (2R,12Z,15Z)-1-(octyloxy)henicosa-12,15-dien-2-ol. LC/MS (m+H)=437.6.

12

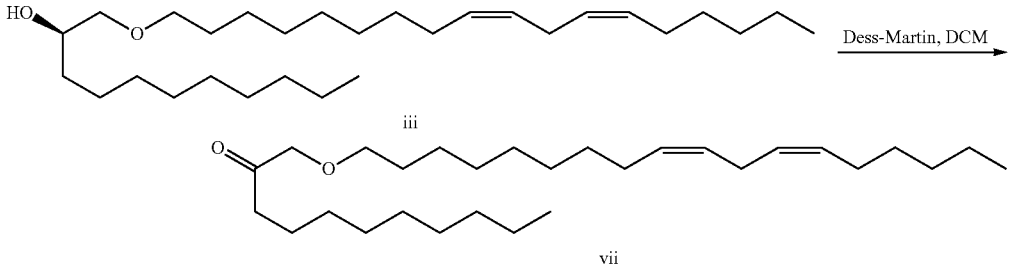


The alcohol was carried on to final Compound 12 as described for Compound 1. ^1H NMR ($CDCl_3$, 300 mHz) δ 0.85-0.091 (m, 6H), 1.272 (s, 34H), 1.46 (m, 1H), 1.57 (m, 1H), 1.65 (s, 4H), 2.01-2.08 (3H), 2.30 (m, 6H), 2.52 (m, 1H), 2.75-2.79 (m, 2H), 3.29-3.4 (m, 2H), 3.46-3.51 (dd, J=9.76 Hz, 1H), 5.30-5.39 (m, 4H); LC/MS (m+H)=464.7.

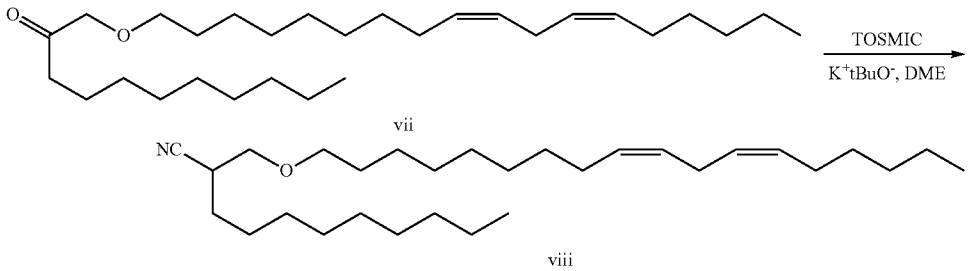
Compounds 13-16 are novel cationic lipids and were prepared according to General Scheme 2 above.

Compound	Structure	LC/MS (m + 1)
13		492.7
14		436.7
15		577.0
16		506.8

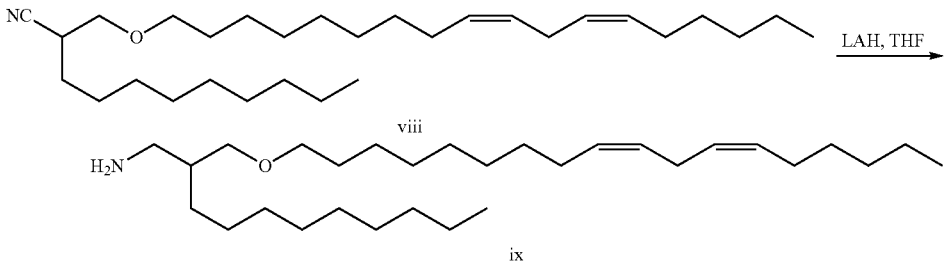
N,N-dimethyl-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}undecan-1-amine (Compound 17)



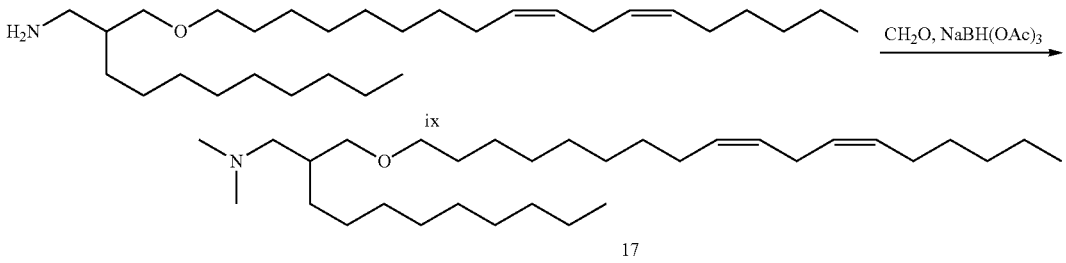
To a solution of alcohol iii (15 g, 34.3 mmol) in dichloromethane (50 mL) was added Dess-Martin Periodinane (14.6 g, 343 mmol) and the reaction was stirred at ambient temperature for 16 hours. The solids were filtered and the filtrate partitioned between water/DCM. The organics were dried over sodium sulfate, filtered and evaporated, in vacuo. Purification by flash chromatography (silica, 0-15% ethyl acetate/hexanes) gave ketone vii. LC/MS (M+H)=435.6.



To a solution of ketone vii (10 g, 23.0 mmol) in DME (40 mL) was added TOSMIC (5.8 g, 29.9 mmol) and the solution was cooled to 0° C. To the cooled solution was added potassium tert-butoxide (46 mmol, 46 mL of a 1M solution in tBuOH) dropwise. After 30 minutes the reaction was partitioned between hexanes and water. The organics were dried over sodium sulfate, filtered and evaporated in vacuo. Purification by flash chromatography (silica, 0-10% ethyl acetate/hexanes) gave nitrile viii. LC/MS (M+H) =446.6.



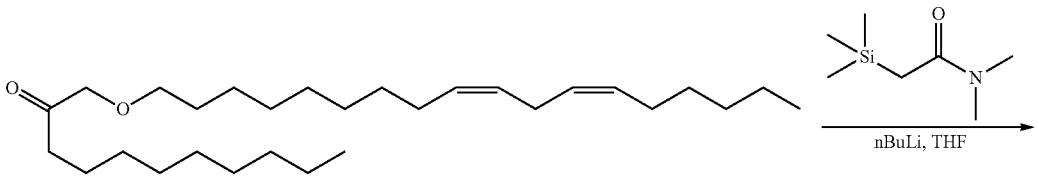
To a solution of nitrile viii (4.6 g, 10.4 mmol) in THF (25 mL) was added lithium aluminum hydride (0.8 g, 20.7 mmol) at ambient temperature. The reaction was quenched with sodium sulfate decahydrate solution and the solids were filtered. The filtrate was dried over sodium sulfate, filtered and evaporated in vacuo to give crude amine ix which was carried directly into next reaction, LC/MS (M+H)=450.6.



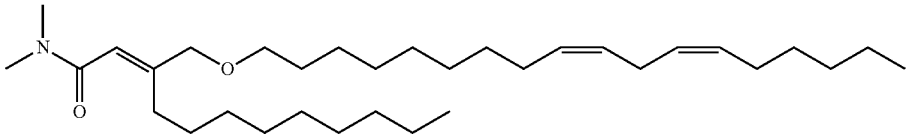
17

A solution of amine ix (4.7 g, 10.3 mmol) and formaldehyde (2.5 g, 31.1 mmol) in THF (25 mL) was treated with sodium, triacetoxyborohydride (6.6%, 31.1 mmol) at ambient temperature. After aging for 15 minutes, the reaction was quenched with 1M sodium hydroxide and partitioned between water and hexanes. The organics were dried over sodium sulfate, filtered and evaporated in vacuo. Purification by preparative reverse phase chromatography (C8 column, acetonitrile/water gradient) gave compound 17. LC/MS (M+H)=479.6. ^{1}H NMR ($CDCl_3$, 400 mHz) δ 5.36 (m, 4H), 3.38 (m, 3H), 3.26 (m, 1H), 2.75 (t, J=6.4 Hz, 2H), 2.22 (m, 1H), 2.19 (s, 6H), 2.04 (m, 5H), 1.71 (m, 1H), 1.54 (m, 2H), 1.28 (m, 32H), 0.83 (m, 6H).

N,N-dimethyl-3-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}dodecan-1-amine (Compound 18)

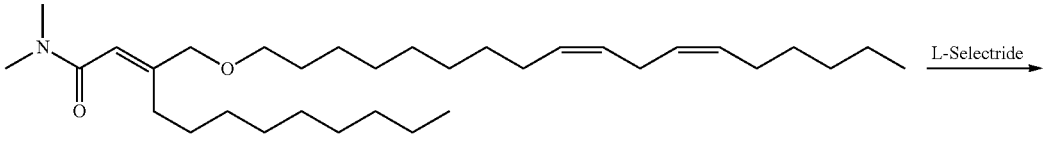


vii

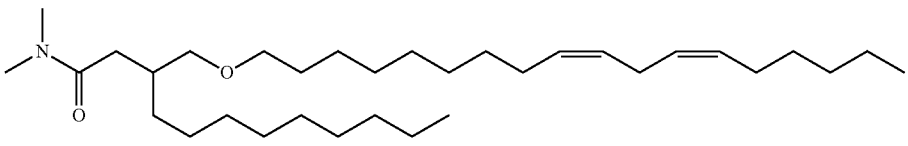


xi

A solution of silyl amide (12.4 g, 78 mmol) in THF (50 mL) was cooled to −78° C. and treated with nBuLi (62.4 mmol, 25 mL of a 2.5M solution) and aged for 10 minutes. To this solution was transferred ketone vii (12 g, 27.6 mmol) in a small portion of dry THF. The reaction was aged 15 minutes then warmed to ambient temperature, quenched with sodium bicarbonate solution and partitioned between water and hexanes. The organics were dried over sodium sulfate, filtered and evaporated in vacuo to give amide xi LC/MS (M+H)=505.6.

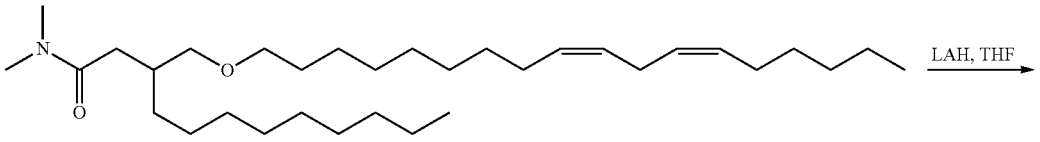


xi

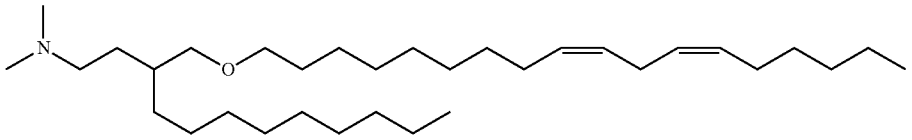


xii

Amide xi (7 g, 13.9 mmol) was treated with L-Selectride (55.6 mmol, 55.6 mL of a 1M solution) in a microwave vial. The reaction was sealed and irradiated in a microwave reaction set at 70° C. for 16 hours. The reaction was then diluted with dichloromethane and quenched by careful addition of sodium perborate solid until effervescence stopped. The solids were filtered and the filtrate evaporated in vacuo to give xii. LC/MS (M+H)=507.6.



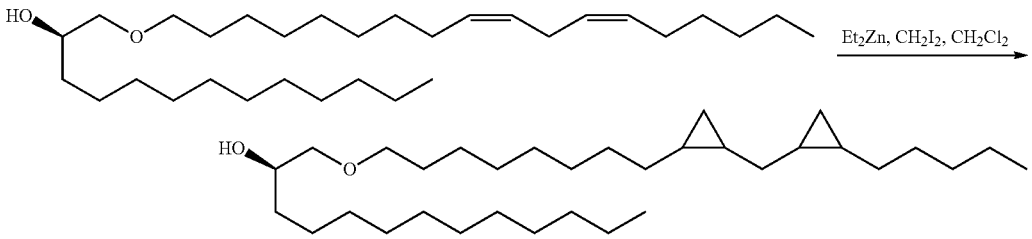
xii



18

To a solution of amide xii (7 g, 13.8 mmol) in THF (30 mL) was added lithium aluminum hydride (1.1 g, 27.7 mmol). The reaction was quenched with sodium sulfate decahydrate solution and the solids filtered. The organics were evaporated in vacuo and the product purified by preparative reverse phase chromatography (CS column, acetonitrile/water gradient) to give compound 18. LC/MS (M+H)=493.6. ^{1}H NMR ($CDCl_3$, 400 mHz) δ 5.38 (m, 4H), 3.38 (m, 2H), 3.26 (m, 2H), 2.78 (t, J=6.4 Hz, 2H), 2.25 (m, 8H), 2.04 (m, 4H), 1.56 (m, 4H), 1.29 (m, 32H), 0.89 (m, 6H).

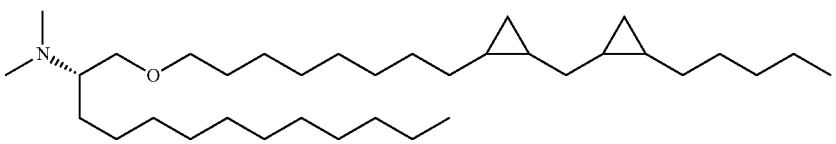
(2S)—N,N-dimethyl-1-[(8-{2-[(2-pentylcyclopropyl)methyl]cyclopropyl}octyl)oxy]tridecan-2-amine (Compound 19)



xiv

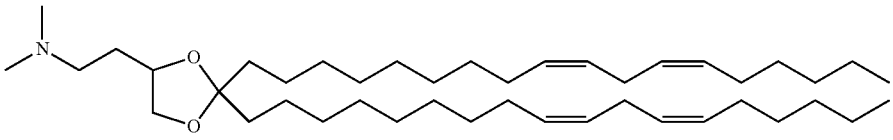
A solution of diene (24 g, 51.6 mmol) in dichloromethane (100 mL) was cooled to −15° C. To this solution was added diethyl zinc (310 mmol, 310 mL of a 1M solution) followed by diiodomethane (25 mL, 310 mmol) and the reaction was aged for 16 hours while slowly warming to ambient temperature. The reaction was quenched with ammonium chloride solution and partitioned between water and dichloromethane. The organics were dried over sodium sulfate, filtered and evaporated in vacuo. Purification by flash chromatography (silica, 0-25% ethyl acetate/hexanes) gave biscyclopropane intermediate xiv. LC/MS (M+H)=493.6.

19



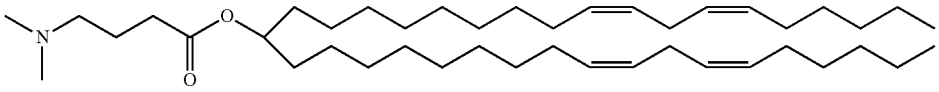
Compound xiv was carried on to final compound 19 as outlined for compound 1 above, LC/MS (M+H)=520.8.

Compound 20 is DLinKC2DMA as described in *Nature Biotechnology*, 2010, 28, 172-176, WO 2010/042877 A1, WO 2010/048536 A2, WO 2010/088537 A2, and WO 2009/127060 A1.



(20)

Compound 21 is MC3 as described in WO 2010/054401, and WO 2010/144740 A1.



(21)

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) are 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 contains 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 9-1.2 mg/mL in an alcohol, (for example ethanol). The ratio of the lipids has a mole percent range of 25-98 for the cationic lipid with a target of 35-65, the helper lipid has a mole percent range from 0-75 with a target of 30-50, the PEG lipid has a mole percent range from 1-1.5 with a target of 1-6, and the DSPC has a mole percent range of 0-15 with a target of 0-12. The siRNA solution contains 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 has 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 has effect of controlling the particle size of the LNPs between 30 and 200 nm. The solution is then 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 uses 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 uses a tangential flow filtration format (TFF). This process uses a membrane nominal molecular weight cutoff range from 30-500 KD. The membrane format can be hollow fiber or flat sheet cassette. The TFF processes with the proper molecular weight cutoff retains 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 is then 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 Mass.) 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. Tire assay is performed using a SpectraMax M5e microplate spectropho-

tometer from Molecular Devices (Sunnyvale, Calif.). Samples are excited at 485 nm and fluorescence emission was 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, N.Y.). 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, N.Y.) 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 are determined by Reverse Phase High-Performance liquid Chromatography (RP-HPLC) using Waters 2695 Alliance system (Water Corporation, Milford Mass.) with a Corona charged aerosol detector (CAD) (ESA Biosciences, Inc, Chelmsford, Mass.). Individual lipids in RDVs are analyzed, using an Agilent Zorbax SB-CIS (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 changes 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 were 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'-UUU*A*U*UCC*GA*U*A*C*UUCUC*UAU-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-CUUUAACAAUUCCU*GAAAU***TsT**-iB-3' (SEQ ID NO.: 3)

3'-UsU*GAAAUUG*UUAAGGACUsUsUsA-5' (SEQ ID NO.: 4)

AUGC - Ribose

iB - Inverted deoxy abasic

UC - 2' Fluoro

AGT - 2' Deoxy

AGU - 2' OCH_3

UsA - phophorothioate linkage

Example 1

Mouse In Vivo Evaluation of Efficacy

LNPs utilizing Compounds 1-12, in the nominal compositions described immediately above, were evaluated for in vivo efficacy. The siRNA targets 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 organotropic 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 are measured prior to administration of the RDVs. Luciferin in PBS (15 mg/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 were tail vein injected n a volume of 0.2 mL. Final dose levels ranged from 0.1 to 0.5 mg/kg siRNA. PBS vehicle alone was dosed as a control. Mice were 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. Systemic administration of the luciferase siRNA RDVs decreased luciferase expression in a dose dependant manner. Greater efficacy was observed in mice dosed with Compound 1 containing RDVs than with the RDV containing the octyl-CLinDMA (OCD) cationic lipid (FIG. **1**). OCD is known and described in WO2010/021865.

Example 2

Rat In Vivo Evaluation of Efficacy and Toxicity

LNPs utilizing compounds in the nominal compositions described above, were 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 were tail vein injected in a volume of 1 to 1.5 ml. Infusion rate is approximately 3 ml/min. Five rats were 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 are taken and placed in RNALater (Ambion) for mRNA analysis. Preserved liver tissue was 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 were determined by quantitative RT-PCR. Message was amplified from purified RNA utilizing a rat ApoB commercial probe set (Applied Biosystems Cat #RN01499054_ml). The PCR reaction was 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 were determined by RT-PCR using a commercial probe set (Applied Biosytems Cat. No. Mm00478295_ml). 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 were performed on the Siemens Advia 1800 Clinical Chemistry Analyzer utilizing the Siemens alanine aminotransferase (Cat#03039631) and aspartate aminotransferase (Cat#03039631) reagents. Similar efficacy was observed in rats dosed with Compound 1 containing RDV than with the RDV containing the cationic lipid DLinKC2DMA (Compound 20) or MC3 (Compound 21, FIG. **2**). Additionally, 3 out of 4 rats treated with 3 mg/kg DLinKC2DMA (Compound 20) failed to survive 48 hours and 2 out of 4 rats treated with 3 mg/kg MC3 (Compound 21) failed to survive 48 hours, 1 out of 4 rats treated with 10 mg/kg Compound 1 survived at 48 hours post dose.

Example 3

Determination of Cationic Lipid Levels in Rat Liver

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

Standards were 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 was mixed with 300 μL of extraction/protein precipitating solvent (50/50 acetonitrile/methanol containing 500 nM internal standard) and the plate was centrifuged to sediment precipitated protein. A volume of 200 μL of each supernatant was transferred to separate wells of a 96-well plate and 10 μl of each standard was directly analyzed by LC/MS-MS.

Absolute quantification versus standards prepared and extracted from liver homogenate was 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 was injected onto a RDS Hypersil C8 HPLC column (Thermo, 50×2 mm, 3 μm) at ambient temperature.

Mobile Phase A:

95% H_2O/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 was 0.5 mL/min and gradient elution profile was 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 runtime was 5.8 min. API 4000 source parameters were CAD: 4, CUR: 15, GS1: 65, GS2: 35, IS: 4000, TEM: 550, CXP: 15, DP: 60, EP: 10. In rats dosed with Compound 1 containing RDV liver levels were lower than with the RDV containing the cationic lipid DLinKC2DMA (Compound 20) or MC3 (Compound 21, FIG. **3**).

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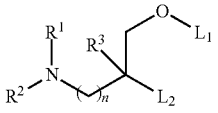
What is claimed is:

1. A cationic lipid of Formula A:

wherein:

A



R^1 and R^2 are independently selected from H, (C_1-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';

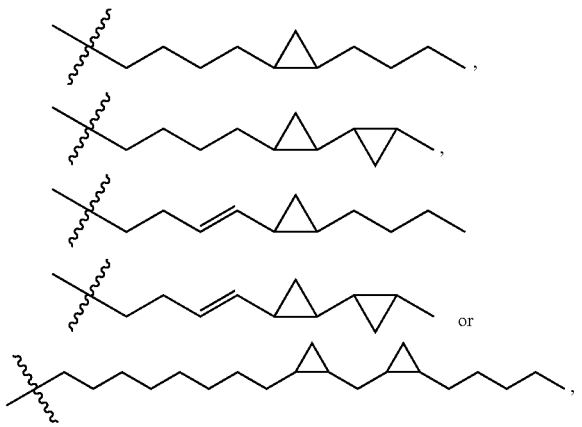
R^3 is selected from H and (C_1-C_6)alkyl, said alkyl optionally substituted with one to three substituents selected from R';

R' is independently selected from halogen, R", OR", SR", CN, CO_2R" and 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; and

one of L_1 and L_2 is



and the other is C_3-C_{24} alkyl and C_3-C_{24} alkenyl, said alkyl and alkenyl are optionally substituted with one or more substituents selected from R';

or any pharmaceutically acceptable salt or stereoisomer thereof.

2. The cationic lipid of claim **1**, wherein R_1 and R_2 are independently selected from the group consisting of H, methyl, ethyl and propyl.

3. The cationic lipid of claim **2**, wherein R_1 and R_2 each are methyl.

4. The cationic lipid of claim **3**, wherein R_3 is H or methyl.

5. The cationic lipid of claim **4**, wherein R_3 is H.

6. The cationic lipid of claim **1**, wherein n is 0, 1 or 2.

7. The cationic lipid of claim **6**, wherein n is 0 or 1.

8. The cationic lipid of claim **7**, wherein n is 0.

9. A lipid nanoparticle comprising a cationic lipid of claim **1**.

10. The lipid nanoparticle of claim **9**, wherein the lipid nanoparticle further comprises an oligonucleotide.

11. The lipid nanoparticle of claim **10**, wherein the oligonucleotide is an siRNA or miRNA.

12. The lipid nanoparticle of claim **11**, wherein the oligonucleotide is an siRNA.

13. The lipid nanoparticle of claim **9**, wherein the lipid nanoparticle further comprises cholesterol and PEG-DMG.

14. The lipid nanoparticle of claim **9**, wherein the lipid nanoparticle further comprises cholesterol, PEG-DMG and DSPC.

15. The lipid nanoparticle of claim **9**, wherein the lipid nanoparticle further comprises cholesterol, PEG-C-DMA and DSPC.

* * * * *