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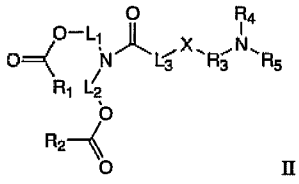
(54) Title: IONIZABLE CATIONIC LIPID FOR RNA DELIVERY



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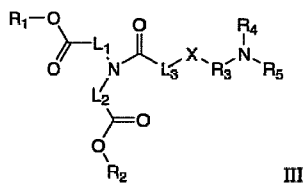

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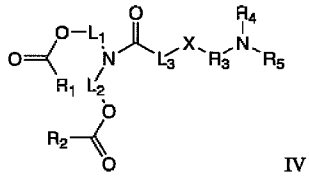

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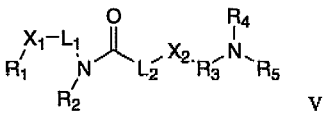

V

(57) Abstract: What are described are compounds of formulas II, HI, IV, and V. The compound of formula II consists of a compound in which R_1 and R_2 are both a linear alkyl consisting of 1 to 12 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons; L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N; X is S; L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or farming a heterocycle with N; R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and R_4 and R_5 are the same or different, each consisting of hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons. The compound of formulas HI and IV consists of a compound in which R_1 consists of a branched alkyl with 12 to 20 carbons; R_2 consists of a linear alkyl with 5 to 10 carbons or a branched alkyl with 12 to 20 carbons; L_1 and L_2 each consists of a bond or a linear alkyl having 1 to 3 carbon atoms; X consists of S or O; L_3 consists of a bond or a lower alkyl; R_3 consists of a lower alkyl; and R_4 and R_5 are the same or different, each consisting of a lower alkyl. The compound of formula V consists of a compound in which R_1 consists of a linear or branched alkyl consisting of 1-18 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons, or a cholesteryl; R_2 consists of a 606338680v1 linear or branched alkyl or an alkenyl consisting of 1 to 18 carbons; L_1 consists of a linear alkyl consisting of 5 to 9 carbons or, when R_1 consists of a cholesteryl then L_1 consists of a linear alkylene or alkenyl consisting of 3 to 4 carbons; X_1 consists of -0-(CO)— or -(CO)-0—; X_2 consists of S or O; La consists of a bond or a linear alkylene of 1 to 6 carbons; R_3 consists of a linear

[Continued on next page]

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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or branched alkylene with 1 to 6 carbons; and R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl of 1 to 6 carbons. The compound of formulas II, III, IV, and V may comprise a pharmaceutically acceptable salt thereof.

IONIZABLE CATIONIC LIPID FOR RNA DELIVERY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This applications claims priority to continuation-in-part applications U.S. Patent Application Number 14/707,796, filed May 8, 2015 and U.S. Patent Application Number 14/707,876, filed May 8, 2015, and U.S. Patent Application No. 14/546,105, filed November 18, 2014.

BACKGROUND

[0002] A number of different types of nucleic acids are currently being developed as therapeutics for the treatment of a number of diseases. These nucleic acids include DNA in gene therapy, plasmids-based interfering nucleic acids, small interfering nucleic acids for use in RNA interference (RNAi), including siRNA, miRNA, antisense molecules, ribozymes and aptamers. As these molecules are being developed, there has been developed a need to produce them in a form that is stable and has a long shelf-life and that can be easily incorporated into an anhydrous organic or anhydrous polar aprotic solvent to enable encapsulations of the nucleic acids without the side-reactions that can occur in a polar aqueous solution or nonpolar solvents.

[0003] The present invention relates to novel lipid compositions that facilitate the intracellular delivery of biologically active and therapeutic molecules. The present invention relates also to pharmaceutical compositions that comprise such lipid compositions, and that are useful to deliver therapeutically effective amounts of biologically active molecules into the cells of patients.

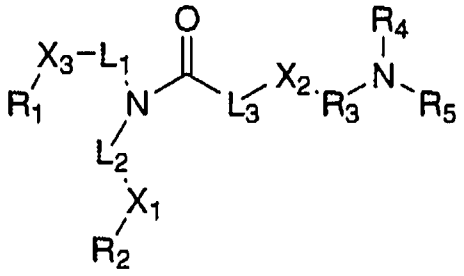
[0004] The delivery of a therapeutic compound to a subject is important for its therapeutic effects and usually it can be impeded by limited ability of the compound to reach targeted cells and tissues. Improvement of such compounds to enter the targeted cells of tissues by a variety of means of delivery is crucial. The present invention relates the novel lipids, in compositions and methods for preparation that facilitate the targeted intracellular delivery of biological active molecules.

[0005] Examples of biologically active molecules for which effective targeting to a patient's tissues is often not achieved include: (1) numerous proteins including immunoglobin proteins, (2) polynucleotides such as genomic DNA, cDNA, or mRNA (3) antisense polynucleotides; and (4) many low molecular weight compounds, whether synthetic or naturally occurring, such as the peptide hormones and antibiotics.

[0006] One of the fundamental challenges now facing medical practitioners is that a number of different types of nucleic acids are currently being developed as therapeutics for the treatment of a number of diseases. These nucleic acids include DNA in gene therapy, plasmids, small interfering nucleic acids (siNA), siRNA, and microRNA (miRNA) for use in RNA interference (RNAi), antisense molecules, ribozymes, antagomirs, and aptamers. As these nucleics are being developed, there is a need to produce lipid formulations that are easy to make and can be readily delivered to a target tissue.

SUMMARY

[0007] What is described herein are compounds of formula I, II, III, IV, and V. What is described is a compound of formula I



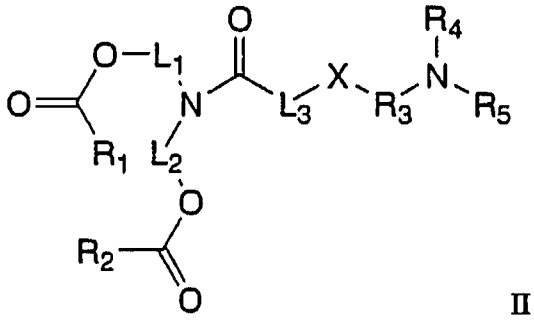
I

in which

R_1 and R_2 each consist of a linear alkyl consisting of 1 to 9 carbons, an alkenyl or alkynyl consisting of 2 to 11 carbons; L_1 and L_2 each consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N; X_1 and X_3 both consist of –CO–O–; X_2 consists of S or O; L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or forming a heterocycle with N; R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and R_4 and R_5 are the same or different, each consisting of a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

[0008] What is also described herein is a compound of Formula II



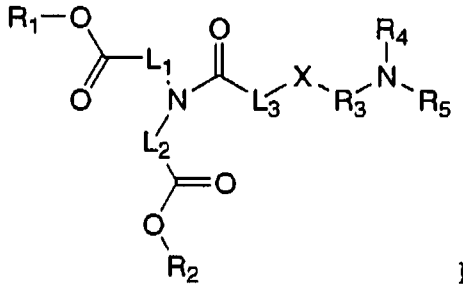
II

in which

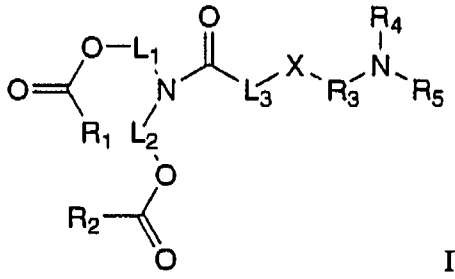
R_1 and R_2 both consist of a linear alkyl consisting of 1 to 12 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons; L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or form a heterocycle with N; X consists of S; L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or forms a heterocycle with N; R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and R_4 and R_5 are the same or different, each consisting of a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons;
or a pharmaceutically acceptable salt thereof.

[0009] In one embodiment of the compound of formula II, L_1 and L_2 both consist of a linear alkylene consisting of five carbons. In another embodiment of the compound of formula I, R_3 is ethylene or propylene. In another embodiment of the compound of formula I, R_4 and R_5 are the same or different, each hydrogen, methyl, or ethyl. In another embodiment of the compound of formula I, L_3 is a bond. In another embodiment of the compound of formula I, R_1 and R_2 both consist of a linear alkenyl consisting of ten carbons.

[0010] What is also described herein is a compound of formula III or IV



III



IV

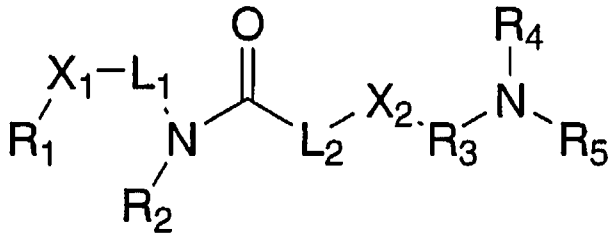
in which

R_1 consists of a branched alkyl with 12 to 20 carbons; R_2 consists of a linear alkyl with 5 to 10 carbons or a branched alkyl with 12 to 20 carbons; L_1 and L_2 each consist of a bond or a linear alkyl having 1 to 3 carbon atoms; X consists of S or O; L_3 consists of a bond or a lower alkyl; R_3 is a lower alkyl, and

R_4 and R_5 are the same or different, each consisting of a lower alkyl; or a pharmaceutically acceptable salt thereof.

[0011] In one embodiment of the compound of formula III or IV, L_3 is consists of a bond. Another embodiment of the compound of formula III or IV, X is S. In another embodiment of the compound of formula III or IV, R_3 is ethylene. In another embodiment of the compound of formula III or IV, R_3 is n-propylene or isopropylene. In another embodiment of the compound of formula III or IV, R_4 and R_5 are separately methyl, ethyl, or isopropyl. In another embodiment of the compound of formula III or IV, L_1 and L_2 each consist of a bond. In another embodiment of the compound of formula III or IV, L_1 and L_2 each consist of a methylene. In another embodiment of the compound of formula III or IV, R_1 and R_2 each consist of branched alkyl. In another embodiment of the compound of formula III or IV, R_2 consists of an alkyl. In another embodiment of the compound of formula III or IV, R_1 and R_2 each consists of 19 or 20 carbon atoms. In another embodiment of the compound of formula III or IV, R_1 or R_2 each consists of 13 or 14 carbon atoms. In another embodiment of the compound of formula III or IV, L_3 consists of methylene, R_3 is ethylene, X_2 is S, and R_4 and R_5 are each methyl. In another embodiment of the compound of formula III or IV, wherein L_3 consists of a bond, R_3 is ethylene, X is S, and R_4 and R_5 are each methyl. In another embodiment of the compound of formula III or IV, L_3 consists of a bond, R_3 consists of n-propylene, X consists of S, and R_4 and R_5 each consist of methyl. In another embodiment of the compound of formula III or IV, L_3 consists of a bond, R_3 consists of isopropylene, X consists of S, and R_4 and R_5 each consist of methyl.

[0012] What is also described is a compound of formula V



V

in which

R_1 consists of a linear or branched alkyl consisting of 1-18 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons, or a cholesteryl; R_2 consists of a linear or branched alkyl or an alkenyl consisting of 1 to 18 carbons; L_1 consists of a linear alkyl consisting of 5 to 9 carbons or, when R_1 consists of a cholesteryl, then L_1 consists of a linear alkylene or alkenyl consisting of 3 to 4 carbons; X_1 consists of –O-(CO)— or –(CO)-O—; X_2 consists of S or O; L_2 consists of a bond or a linear alkylene of 1 to 6 carbons; R_3 consists of a linear or branched alkylene with 1 to 6 carbons; and R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

[0013] In one embodiment of the compound of formula V, L_2 consists of a bond. In another embodiment of the compound of formula V, X_2 consists of S. In another embodiment of the compound of formula V, X_1 is –O-(CO)—. In another embodiment of the compound of formula V, R_3 is ethylene. In another embodiment of the compound of formula V, R_3 is n-propylene or isopropylene. In another embodiment of the compound of formula V, R_4 and R_5 are separately methyl, ethyl, or isopropyl. In another embodiment of the compound of formula V, L_2 consist of a methylene. In another embodiment of the compound of formula V, R_1 and R_2 each consist of branched alkyl. In another embodiment of the compound of formula V, R_2 consists of an alkyl. In another embodiment of the compound of formula V, R_1 and R_2 each consists of 19 or 20 carbon atoms. In another embodiment of the compound of formula V, R_1 or R_2 each consists of 13 or 14 carbon atoms. In another embodiment of the compound of formula V, L_2 consists of methylene, R_3 is ethylene, X_1 is –O-(CO)—, X_2 is S, and R_4 and R_5 are both methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 is ethylene, X_1 is –O-(CO)—, X_2 is S, and R_4 and R_5 are both methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 is n-propylene, X_1 is –O-(CO)—, X_2 is S, and R_4 and R_5 are both methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 is isopropylene, X_1 is –O-(CO)—, X_2 is S, and R_4 and R_5 are both methyl.

[0014] The nucleic acid preferably has an activity of suppressing the expression of a target gene. The target gene preferably is a gene associated with inflammation.

[0015] What is also described herein is a method for introducing a nucleic acid into a cell of a mammal by using any of the compositions, above. The cell may be in a liver, lung, kidney, brain, blood, spleen, or bone. The composition preferably is administered intravenously, subcutaneously, intraperitoneally, or intrathecally. Preferably, the compositions described herein are used in a method for treating cancer or inflammatory disease. The disease may be one selected from the group consisting of immune disorder, cancer, renal disease, fibrotic disease, genetic abnormality, inflammation, and cardiovascular disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1 shows the knockdown activity of siRNA encapsulated by different cationic lipids. The lipids include MC3 (0.3 mg/kg), NC1 (0.3 mg/kg), ATX-547 (0.3 mg/kg), ATX-001 (0.3 and 1.0 mg/kg), ATX-002 (0.3 and 1.0 mg/kg), and ATX-003 (0.3 and 1.0 mg/kg). The amount of Factor VII knockdown in mouse plasma is shown following administration of the siRNA formulation to C57BL6 mice, compared to injection of vehicle alone. The amount of Factor VII in abnormal and normal human plasma is included as a control. Statistically significant decreases in Factor VII levels ($p<0.01$) is shown by an asterisk (*).

[0017] Fig. 2 shows an evaluation of the effect of siRNA of Factor VII activity based on the results shown in Fig. 2, and normalized to percentage knockdown compared to the vehicle alone.

[0018] Fig. 3 shows the knockdown activity of siRNA encapsulated by different cationic lipids. The lipids include MC3 (0.3 and 1.5 mg/kg), NC1 (0.3 mg/kg), AT547 (0.1 and 0.3 mg/kg), AT004 (0.3), AT006 (0.3 and 1.0 mg/kg), ATX-010 (0.3 mg/kg), and AT001 (0.3 and 1.5 mg/kg). The amount of Factor VII knockdown in mouse plasma is shown following administration of the siRNA formulation to C57BL6 mice, compared to injection of vehicle alone. The amount of Factor VII in abnormal and normal human plasma is included as a control. Statistically significant decreases in Factor VII levels ($p<0.01$) is shown by an asterisk (*).

[0019] Fig. 4 shows an evaluation of the effect of siRNA of Factor VII activity based on the results shown in Fig. 2, and normalized to percentage knockdown compared to the vehicle alone.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0020] "At least one" means one or more (*e.g.*, 1-3, 1-2, or 1).

[0021] "Composition" includes a product comprising the specified ingredients in the specified amounts, as well as any product that results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

[0022] "In combination with" as used to describe the administration of a compound of formulas 1, I, and II with other medicaments in the methods of treatment of this invention, means-that the compounds of formulas 1, I, and II and the other medicaments are administered sequentially or concurrently in separate dosage forms, or are administered concurrently in the same dosage form.

[0023] "Mammal" means a human or other mammal, or means a human being.

[0024] "Patient" includes both human and other mammals, preferably human.

[0025] "Alkyl" means a saturated or unsaturated, straight or branched, hydrocarbon chain. In various embodiments, the alkyl group has 1-18 carbon atoms, *i.e.* is a C_1-C_{18} group, or is a C_1-C_{12} group, a C_1-C_6 group, or a C_1-C_4 group. Independently, in various embodiments, the alkyl group has zero branches (*i.e.*, is a straight chain), one branch, two branches, or more than two branches." Alkenyl" means an unsaturated alkyl that may have one double bond, two double bonds, more than two double bonds. "Alkynal" means an unsaturated alkyl that may have one triple bond, two triple bonds, or more than two triple bonds. Alkyl chains may be optionally substituted with 1 substituent (*i.e.*, the alkyl group is mono-substituted), or 1-2 substituents, or 1-3 substituents, or 1-4 substituents, etc. The substituents may be selected from the group consisting of hydroxy, amino, alkylamino, boronyl, carboxy, nitro, cyano, or halo. When the alkyl group incorporates one or more heteroatoms, the alkyl group is referred to herein as a heteroalkyl group. When the substituents on an alkyl group are hydrocarbons, then the resulting group is simply referred to as a substituted alkyl. In various aspects, the alkyl group including substituents has less than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, or 7 carbons.

[0026] "Lower alkyl" means a group having one to six carbon atoms in the chain which chain may be straight or branched. Non-limiting examples of suitable alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, n-pentyl, and hexyl.

[0027] "Alkoxy" means an alkyl-O-group wherein alkyl is as defined above. Non-limiting examples of alkoxy groups include: methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy and heptoxy. The bond to the parent moiety is through the ether oxygen.

[0028] "Alkoxyalkyl" means an alkoxy-alkyl-group in which the alkoxy and alkyl are as previously described. Preferred alkoxyalkyl comprise a lower alkyl group. The bond to the parent moiety is through the alkyl.

[0029] "Alkylaryl" means an alkyl-aryl-group in which the alkyl and aryl are as previously described. Preferred alkylaryls comprise a lower alkyl group. The bond to the parent moiety is through the aryl.

[0030] "Aminoalkyl" means an NH_2-alkyl-group, wherein alkyl is as defined above, bound to the parent moiety through the alkyl group.

[0031] "Carboxyalkyl" means an HOOC-alkyl-group, wherein alkyl is as defined above, bound to the parent moiety through the alkyl group.

[0032] "Commercially available chemicals" and the chemicals used in the Examples set forth herein may be obtained from standard commercial sources, where such sources include, for example, Acros Organics (Pittsburgh, Pa.), Sigma-Adrich Chemical (Milwaukee, Wis.), Avocado Research (Lancashire, U.K.), Bionet (Cornwall, U.K.), Boron Molecular (Research Triangle Park, N.C.), Combi-Blocks (San Diego, Calif.), Eastman Organic Chemicals, Eastman Kodak Company (Rochester, N.Y.), Fisher Scientific Co. (Pittsburgh, Pa.), Frontier Scientific (Logan, Utah), ICN Biomedicals, Inc. (Costa Mesa, Calif.), Lancaster Synthesis (Windham, N.H.), Maybridge Chemical Co. (Cornwall, U.K.), Pierce Chemical Co. (Rockford, Ill.), Riedel de Haen (Hannover, Germany), Spectrum Quality Product, Inc. (New Brunswick, N.J.), TCI America (Portland, Oreg.), and Wako Chemicals USA, Inc. (Richmond, Va.).

[0033] "Compounds described in the chemical literature" may be identified through reference books and databases directed to chemical compounds and chemical reactions, as known to one of ordinary skill in the art. Suitable reference books and treatise that detail the synthesis of reactants useful in the preparation of compounds disclosed herein, or provide references to articles that describe the preparation of compounds disclosed herein, include for example, "Synthetic Organic Chemistry", John Wiley and Sons, Inc. New York; S. R. Sandler et al, "Organic Functional Group Preparations," 2nd Ed., Academic Press, New York, 1983; H. O. House, "Modern Synthetic Reactions," 2nd Ed., W. A. Benjamin, Inc. Menlo Park, Calif., 1972; T. L. Glichrist, "Heterocyclic Chemistry," 2nd Ed. John Wiley and Sons, New York, 1992; J. March, "Advanced Organic Chemistry: reactions, Mechanisms and Structure," 5th Ed., Wiley Interscience, New York, 2001; Specific and analogous reactants may also be identified through the indices of known chemicals prepared by the Chemical Abstract Service of the American Chemical Society, which are available in most public and university libraries, as well as through online databases (e.g., the American Chemical Society, Washington, D.C.). Chemicals that are

known but not commercially available in catalogs may be prepared by custom chemical synthesis houses, where many of the standard chemical supply houses (such as those listed above) provide custom synthesis services.

[0034] "Halo" means fluoro, chloro, bromo, or iodo groups. Preferred are fluoro, chloro or bromo, and more preferred are fluoro and chloro.

[0035] "Halogen" means fluorine, chlorine, bromine, or iodine. Preferred are fluorine, chlorine and bromine.

[0036] "Heteroalkyl" is a saturated or unsaturated, straight or branched, chain containing carbon and at least one heteroatom. The heteroalkyl group may, in various embodiments, have on heteroatom, or 1-2 heteroatoms, or 1-3 heteroatoms, or 1-4 heteroatoms. In one aspect the heteroalkyl chain contains from 1 to 18 (*i.e.*, 1-18) member atoms (carbon and heteroatoms), and in various embodiments contain 1-12, or 1-6, or 1-4 member atoms. Independently, in various embodiments, the heteroalkyl group has zero branches (*i.e.*, is a straight chain), one branch, two branches, or more than two branches. Independently, in one embodiment, the hetereoalkyl group is saturated. In another embodiment, the heteroalkyl group is unsaturated. In various embodiments, the unsaturated heterolkyl may have one double bond, two double bonds, more than two double bonds, and/or one triple bond, two triple bonds, or more than two triple bonds. Heteroalkyl chains may be substituted or unsubstituted. In one embodiment, the heteroalkyl chain is unsubstituted. In another embodiment, the heteroalkyl chain is substituted. A substituted heteroalkyl chain may have 1 substituent (*i.e.*, by monosubstituted), or may have, *e.g.*, 1-2 substituents, or 1-3 substituents, or 1-4 substituents. Exemplary heteroalkyl substituents include esters (—C(O)—O—R) and carbonyls (—C(O)—).

[0037] "Hydroxyalkyl" means an HO-alkyl-group, in which alkyl is previously defined. Preferred hydroxyalkyls contain lower alkyl. Non-limiting examples of suitable hydroxyalkyl groups include hydroxymethyl and 2-hydroxyethyl.

[0038] "Hydrate" is a solvate wherein the solvent molecule is H_2O.

[0039] "Lipid" means an organic compound that comprises an ester of fatty acid and is characterized by being insoluble in water, but soluble in many organic solvents. Lipids are usually divided into at least three classes: (1) "simple lipids," which include fats and oils as well as waxes; (2) "compound lipids," which include phospholipids and glycolipids; and (3) "derived lipids" such as steroids.

[0040] "Lipid particle" means a lipid formulation that can be used to deliver a therapeutic nucleic acid (*e.g.*, mRNA) to a target site of interest (*e.g.*, cell, tissue, organ, and the like). In preferred embodiments, the lipid particle is a nucleic acid-lipid particle, which is

typically formed from a cationic lipid, a non-cationic lipid (*e.g.*, a phospholipid), a conjugated lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid), and optionally cholesterol. Typically, the therapeutic nucleic acid (*e.g.*, mRNA) may be encapsulated in the lipid portion of the particle, thereby protecting it from enzymatic degradation.

[0041] Lipid particles typically have a mean diameter of from 30 nm to 150 nm, from 40 nm to 150 nm, from 50 nm to 150 nm, from 60 nm to 130 nm, from 70 nm to 110 nm, from 70 nm to 100 nm, from 80 nm to 100 nm, from 90 nm to 100 nm, from 70 to 90 nm, from 80 nm to 90 nm, from 70 nm to 80 nm, or 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 1 15 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic. In addition, nucleic acids, when present in the lipid particles of the present invention, are resistant in aqueous solution to degradation with a nuclease.

[0042] "Solvate" means a physical association of a compound of this disclosure with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like.

[0043] "Lipid encapsulated" can mean a lipid particle that provides a therapeutic nucleic acid such as an mRNA with full encapsulation, partial encapsulation, or both. In a preferred embodiment, the nucleic acid (*e.g.*, mRNA) is fully encapsulated in the lipid particle.

[0044] "Lipid conjugate" means a conjugated lipid that inhibits aggregation of lipid particles. Such lipid conjugates include, but are not limited to, PEG-lipid conjugates such as, *e.g.*, PEG coupled to dialkyloxypropyls (*e.g.*, PEG-DAA conjugates), PEG coupled to diacylglycerols (*e.g.*, PEG-DAG conjugates), PEG coupled to cholesterol, PEG coupled to phosphatidylethanolamines, and PEG conjugated to ceramides, cationic PEG lipids, polyoxazoline (POZ)-lipid conjugates, polyamide oligomers (*e.g.*, ATTA-lipid conjugates), and mixtures thereof. PEG or POZ can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG or the POZ to a lipid can be used including, *e.g.*, non-ester-containing linker moieties and ester-containing linker moieties. In certain preferred embodiments, non-ester-containing linker moieties, such as amides or carbamates, are used.

[0045] "Amphipathic lipid" means the material in which the hydrophobic portion of the lipid material orients into a hydrophobic phase, while the hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of polar or charged groups such as carbohydrates, phosphate, carboxylic, sulfato, amino, sulfhydryl, nitro, hydroxyl, and other like groups. Hydrophobicity can be conferred by the inclusion of apolar groups that include, but are not limited to, long-chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic, or heterocyclic group(s). Examples of amphipathic compounds include, but are not limited to, phospholipids, aminolipids, and sphingolipids.

[0046] Representative examples of phospholipids include, but are not limited to, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoylphosphatidylcholine, and dilinoleoylphosphatidylcholine. Other compounds lacking in phosphorus, such as sphingolipid, glycosphingolipid families, diacylglycerols, and β-acyloxyacids, are also within the group designated as amphipathic lipids. Additionally, the amphipathic lipids described above can be mixed with other lipids including triglycerides and sterols.

[0047] "Neutral lipid" means a lipid species that exist either in an uncharged or neutral zwitterionic form at a selected pH. At physiological pH, such lipids include, for example, diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cholesterol, cerebrosides, and diacylglycerols.

[0048] "Non-cationic lipid" means an amphipathic lipid or a neutral lipid or anionic lipid, and is described in more detail below.

[0049] "Anionic lipid" means a lipid that is negatively charged at physiological pH. These lipids include, but are not limited to, phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol (POPG), and other anionic modifying groups joined to neutral lipids.

[0050] The term "hydrophobic lipid" means a compound having apolar groups that include, but are not limited to, long-chain saturated and unsaturated aliphatic hydrocarbon groups and such groups optionally substituted by one or more aromatic, cycloaliphatic, or heterocyclic

group(s). Suitable examples include, but are not limited to, diacylglycerol, dialkylglycerol, N-N-dialkylamino, 1,2-diacyloxy-3-aminopropane, and 1,2-dialkyl-3-aminopropane.

[0051] The terms "cationic lipid" and "amino lipid" are used interchangeably herein to include those lipids and salts thereof having one, two, three, or more fatty acid or fatty alkyl chains and a pH-titratable amino head group (*e.g.*, an alkylamino or dialkylamino head group). The cationic lipid is typically protonated (*i.e.*, positively charged) at a pH below the pK_a of the cationic lipid and is substantially neutral at a pH above the pK_a. The cationic lipids of the invention may also be termed titratable cationic lipids. In some embodiments, the cationic lipids comprise: a protonatable tertiary amine (*e.g.*, pH-titratable) head group; C_{18} alkyl chains, wherein each alkyl chain independently has 0 to 3 (*e.g.*, 0, 1, 2, or 3) double bonds; and ether, ester, or ketal linkages between the head group and alkyl chains. Such cationic lipids include, but are not limited to, DSDMA, DODMA, DLinDMA, DLenDMA, γ- DLenDMA, DLin-K-DMA, DLin-K-C2-DMA (also known as DLin-C2K-DMA, XTC2, and C2K), DLin-K-C3 -DM A, DLin-K-C4-DMA, DLen-C2K-DMA, y-DLen-C2K-DMA, DLin- M-C2-DMA (also known as MC2), DLin-M-C3 -DMA (also known as MC3) and (DLin-MP- DMA)(also known as 1-Bl 1).

[0052] The term "substituted" means substitution with specified groups other than hydrogen, or with one or more groups, moieties, or radicals which can be the same or different, with each, for example, being independently selected.

[0053] By "antisense nucleic acid", it is meant a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 Nature 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 Science 261, 1004 and Woolf et al., U.S. Pat. No. 5,849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. In addition, antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target RNA. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA

expression vector or equivalent thereof. Antisense RNA is an RNA strand having a sequence complementary to a target gene mRNA. Sense RNA has a sequence complementary to the antisense RNA, and annealed to its complementary antisense RNA to form iNA. These antisense and sense RNAs have been conventionally synthesized with an RNA synthesizer.

[0054] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0055] By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribo-furanose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of an interfering RNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA. As used herein, the terms "ribonucleic acid" and "RNA" refer to a molecule containing at least one ribonucleotide residue, including siRNA, antisense RNA, single stranded RNA, microRNA, mRNA, noncoding RNA, and multivalent RNA. A ribonucleotide is a nucleotide with a hydroxyl group at the 2' position of a ß-D-ribo-furanose moiety. These terms include double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as modified and altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution, modification, and/or alteration of one or more nucleotides. Alterations of an RNA can include addition of non-nucleotide material, such as to the end(s) of an interfering RNA or internally, for example at one or more nucleotides of an RNA nucleotides in an RNA molecule include non-standard

nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs.

[0056] By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar, and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate, and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, supra; Eckstein, et al., International PCT Publication No. WO 92/07065; Usman, et al, International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach, et al, Nucleic Acids Res. 22:2183, 1994. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include: inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.*, 6-methyluridine), propyne, and others (Burgin, et al., Biochemistry 35:14090, 1996; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine, and uracil at 1' position or their equivalents.

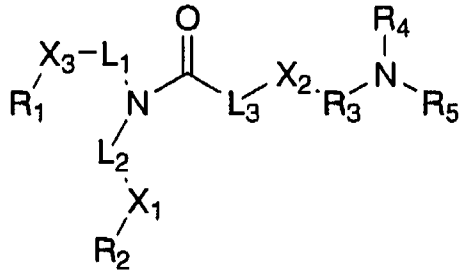
[0057] As used herein complementary nucleotide bases are a pair of nucleotide bases that form hydrogen bonds with each other. Adenine (A) pairs with thymine (T) or with uracil (U) in RNA, and guanine (G) pairs with cytosine (C). Complementary segments or strands of nucleic acid that hybridize (join by hydrogen bonding) with each other. By "complementary" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence either by traditional Watson-Crick or by other non-traditional modes of binding.

[0058] MicroRNAs (miRNA) are single-stranded RNA molecules of 21-23 nucleotides in length, which regulate gene expression miRNAs are encoded by genes that are transcribed from DNA but not translated into protein (non-coding RNA); instead they are processed from primary transcripts known as pri-miRNA to short stem-loop structures called pre-miRNA and finally to functional miRNA. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to downregulate gene expression

[0059] As used herein the term "small interfering RNA (siRNA)", sometimes known as short interfering RNA or silencing RNA, is used to refer to a class of dsRNA molecules, 16-40 nucleotides in length, that play a variety of roles in biology. Most notably, siRNA is involved in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene. In addition to their role in the RNAi pathway, siRNAs also act in RNAi-related pathways, *e.g.*, as an antiviral mechanism or in shaping the chromatin structure of a genome; the complexity of these pathways is only now being elucidated.

[0060] As used herein, the term RNAi refers to an RNA-dependent gene silencing process that is controlled by the RNA-induced silencing complex (RISC) and is initiated by short dsRNA molecules in a cell, where they interact with the catalytic RISC component called argonaute protein. When the dsRNA or RNA-like iNA or siRNA is exogenous (coming from infection by a virus with an RNA genome or from transfected iNA or siRNA), the RNA or iNA is imported directly into the cytoplasm and cleaved to short fragments by an enzyme named Dicer. The initiating dsRNA can also be endogenous (originating in the cell), as in pre-miRNAs expressed from RNA-coding genes in the genome. The primary transcripts from such genes are first processed to form the characteristic stem-loop structure of pre-miRNA in the nucleus, then exported to the cytoplasm to be cleaved by Dicer. Thus, the two dsRNA pathways, exogenous and endogenous, converge at the RISC complex. The active components of RISC, argonaute proteins, cleave the target mRNA strand complementary to their bound siRNA or iNA. As the fragments produced by Dicer are double-stranded, they could each in theory produce a functional siRNA or iNA. However, only one of the two strands, called the guide strand, binds argonaute protein and directs gene silencing. The other anti-guide strand or passenger strand is degraded during RISC activation.

[0061] What is described herein are compounds of formula I, II, III, IV, and V. What is described herein is a compound of Formula I



I

in which

R_1 and R_2 both consist of a linear alkyl consisting of 1 to 9 carbons, an alkenyl or alkynyl consisting of 2 to 11 carbons;

L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N;

X_1 and X_3 both consist of –CO–O–;

X_2 is S or O;

L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or forming a heterocycle with N;

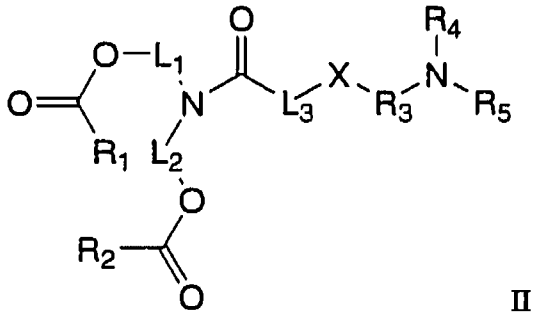
R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and

R_4 and R_5 are the same or different, consisting of a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons,

or pharmaceutically acceptable salts thereof.

[0062] What are also described herein are any of the compounds listed in ATX-001 to ATX-017, ATX-021 to ATX-023, and ATX-026 to ATX-030 listed in Table 1, below, or a pharmaceutically acceptable salt thereof.

[0063] What is also described herein is a compound of Formula II



II

in which

R_1 and R_2 both consist of a linear alkyl consisting of 1 to 12 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons,

L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N,

X is S,

L_3 is a bond or a linear alkylene consisting of 1 to 6 carbons, or forming a heterocycle with N,

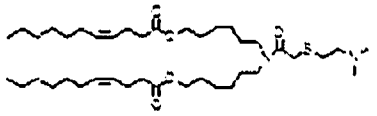
R_3 is a linear or branched alkylene consisting of 1 to 6 carbons, and

R_4 and R_5 are the same or different, each a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons; or a pharmaceutically acceptable salt.

[0064] In one embodiment of the compound of formula II, L_1 and L_2 both consist of a linear alkylene consisting of five carbons. In another embodiment of the compound of formula I, R_3 is ethylene or propylene. In another embodiment of the compound of formula I, R_4 and R_5 are the same or different, each hydrogen, methyl, or ethyl. In another embodiment of the compound of formula I, L_3 is a bond. In another embodiment of the compound of formula I, R_1 and R_2 both consist of a linear alkenyl consisting of ten carbons.

[0065] In another embodiment of the compounds of formulas I and II, the compound consists of a compound selected from any of the compounds listed in ATX-001 to ATX-017, ATX-021 to ATX-023, and ATX-026 to ATX-030 listed in Table 1, below, or formulas 1) to 12), or a pharmaceutically acceptable salt thereof.

1)



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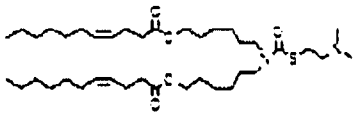

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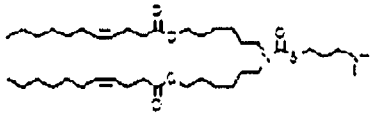

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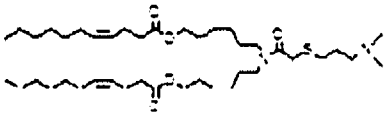

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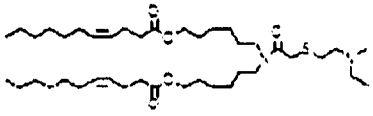

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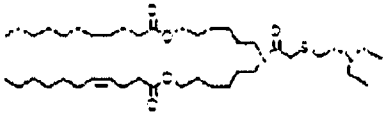

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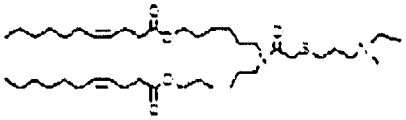

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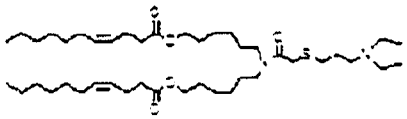

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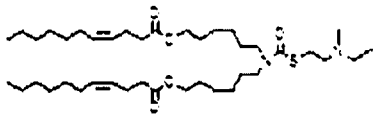
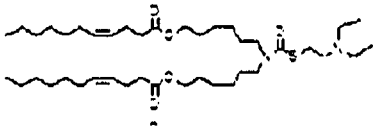


10)



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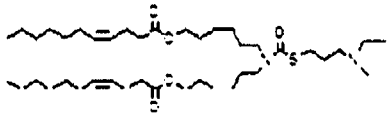
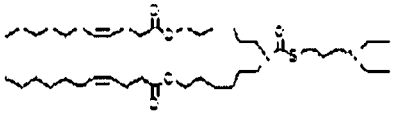
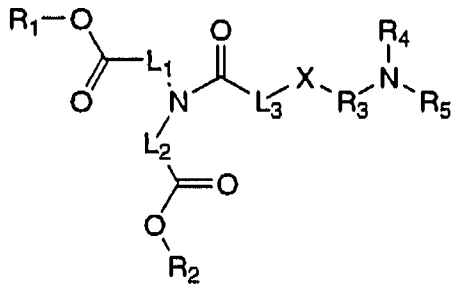


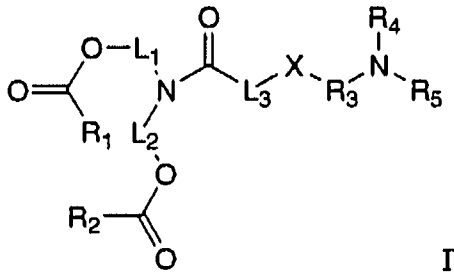
12)



[0066] What is also described herein is a compound of formula III or IV



III



IV

wherein

R_1 consists of a branched alkyl with 12 to 20 carbons,

R_2 consists of a linear alkyl with 5 to 10 carbons or a branched alkyl with 12 to 20 carbons,

L_1 and L_2 each consists of a bond or a linear alkyl having 1 to 3 carbon atoms,

X consists of S or O,

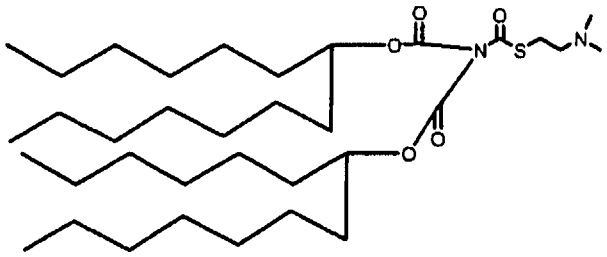
L_3 consists of a bond or a lower alkyl,

R_3 consists of a lower alkyl, and

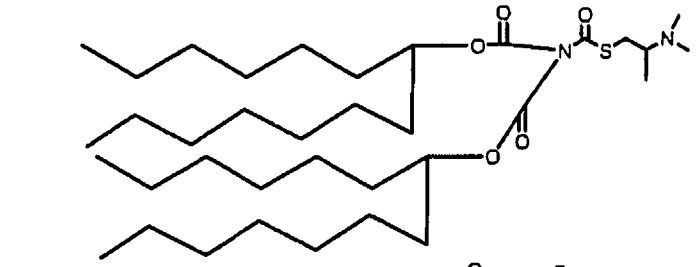
R_4 and R_5 are the same or different, each consisting of a lower alkyl;

or a pharmaceutically acceptable salt thereof.

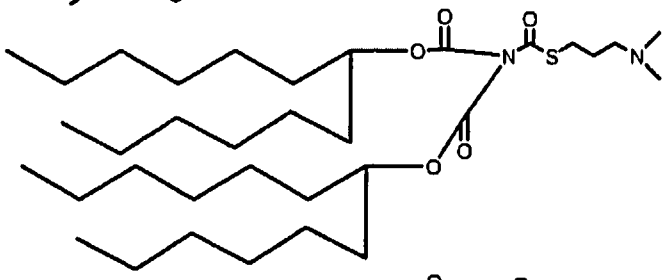
[0067] In one embodiment of the compound of formula III or IV, L_3 consists of a bond. Another embodiment of the compound of formula III or IV, X is S. In another embodiment the compound of formula III or IV, R_3 is ethylene. In another embodiment of the compound of formula III or IV, R_3 is n-propylene or isopropylene. In another embodiment of the compound of formula III or IV, R_4 and R_5 are separately methyl, ethyl, or isopropyl. In another embodiment of the compound of formula III or IV, L_1 and L_2 both consist of a bond. In another embodiment of the compound of formula III or IV, L_1 and L_2 both consist of a methylene. In another embodiment of the compound of formula III or IV, R_1 and R_2 both consist of branched alkyl. In another embodiment of the compound of formula III or IV, R_2 consists of an alkyl. In another embodiment of the compound of formula III or IV, R_1 and R_2 both consist of 19 or 20 carbon atoms. In another embodiment of the compound of formula III or IV, R_1 and R_2 both consist of 13 or 14 carbon atoms. In another embodiment of the compound of formula III or IV, L3 consists of methylene, R_3 consists of ethylene, X_2 consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula III or IV, wherein L_3 consists of a bond, R_3 consists of ethylene, X consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula III or IV, L_3 consists of a bond, R_3 consists of n-propylene, X consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula III or IV, L_3 consists of a bond, R_3 consists of isopropylene, X consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula III or IV, selected from the group consisting of a compound of formula ATX-B-1 to ATX-B-12 as follows.



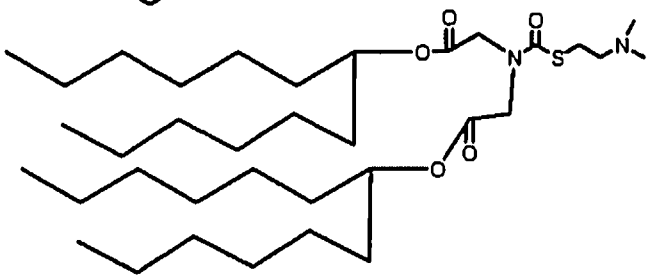
ATX-B-1



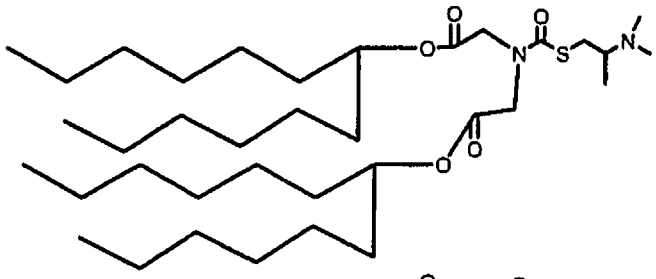
ATX-B-2



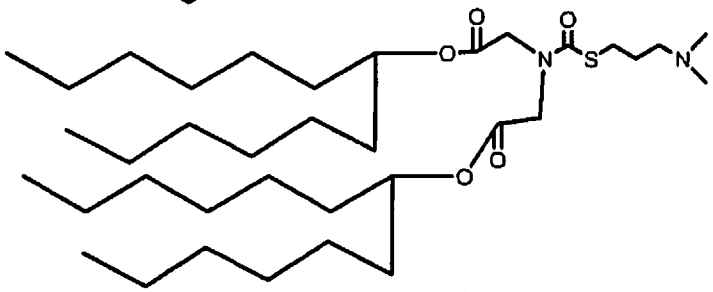
ATX-B-3



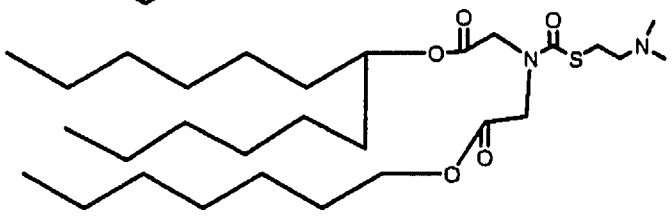
ATX-B-4



ATX-B-5



ATX-B-6



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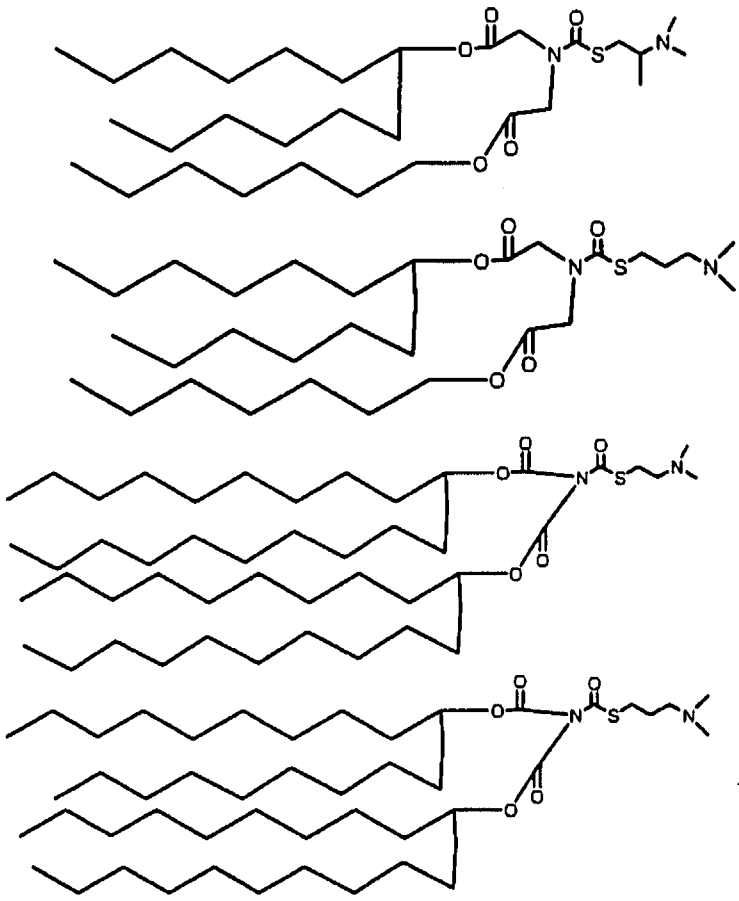


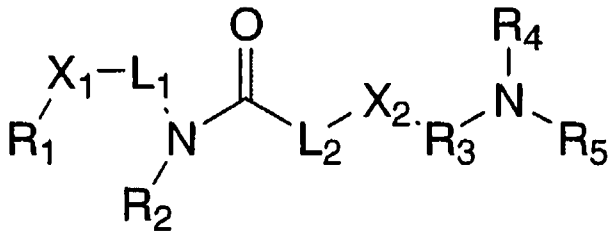
ATX-B-8

ATX-B-9

ATX-B-10

ATX-B-12

What is also described is a compound of formula V



V

wherein

R_1 consists of a linear or branched alkyl consisting of 1-18 carbons, an alkenyl or alkynyl consisting of 2 to 12 carbons, or a cholesteryl;

R_2 consists of a linear or branched alkyl or an alkenyl consisting of 1 to 18 carbons;

L_1 consists of a linear alkyl consisting of 5 to 9 carbons or, when R_1 consists of a cholesteryl then L_1 consists of a linear alkylene or alkenyl consisting of 3 to 4 carbons;

X_1 consists of –O-(CO)— or –(CO)-O—;

X_2 consists of S or O;

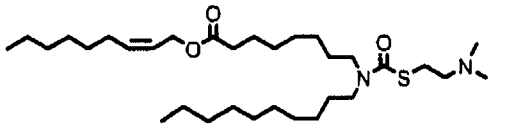
L_2 consists of a bond or a linear alkylene of 1 to 6 carbons;

R_3 consists of a linear or branched alkylene with 1 to 6 carbons; and

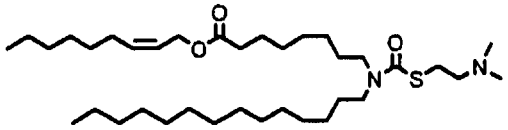
R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof

[0068] In one embodiment of the compound of formula V, L_2 consists of a bond. In another embodiment of the compound of formula V, X_2 consists of S. In another embodiment of the compound of formula V, X_1 is –O-(CO)—. In another embodiment of the compound of formula V, R_3 consists of ethylene. In another embodiment of the compound of formula V, R_3 consists of n-propylene or isopropylene. In another embodiment of the compound of formula V, R_4 and R_5 each consist of methyl, ethyl, or isopropyl. In another embodiment of the compound of formula V, L_2 consists of a methylene. In another embodiment of the compound of formula V, R_1 and R_2 each consist of branched alkyl. In another embodiment of the compound of formula V, R_2 consists of an alkyl. In another embodiment of the compound of formula V, R_1 and R_2 each consists of 19 or 20 carbon atoms. In another embodiment of the compound of formula V, R_1 or R_2 each consists of 13 or 14 carbon atoms. In another embodiment of the compound of formula V, L_2 consists of methylene, R_3 consists of ethylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 is ethylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 consists of n-propylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula V, L_2 consists of a bond, R_3 consists of isopropylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl. In another embodiment of the compound of formula V, the compound is selected from the compounds of formula ATX-A-1 to ATX-A-22



ATX-A-1



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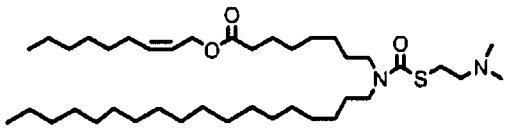

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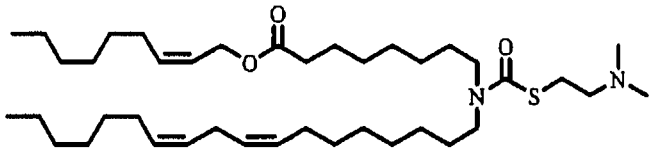

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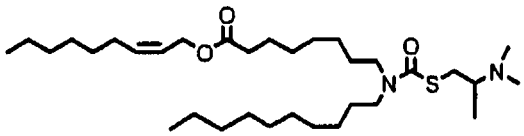

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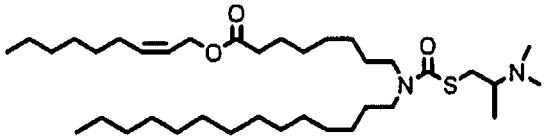

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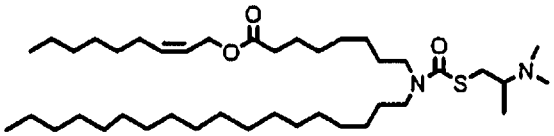

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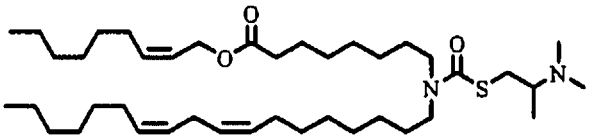

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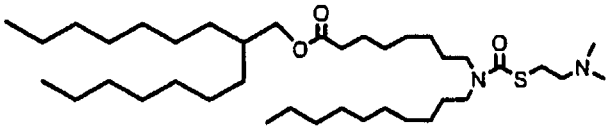

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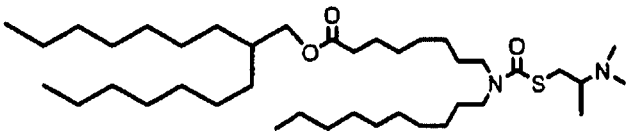

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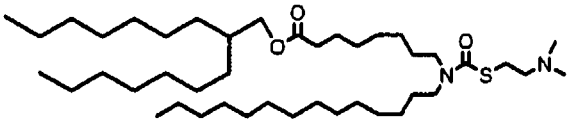

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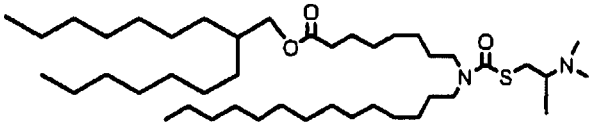

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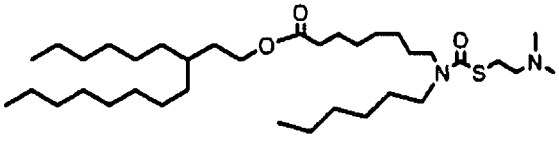

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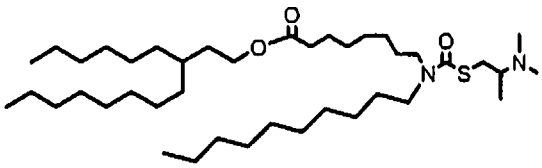

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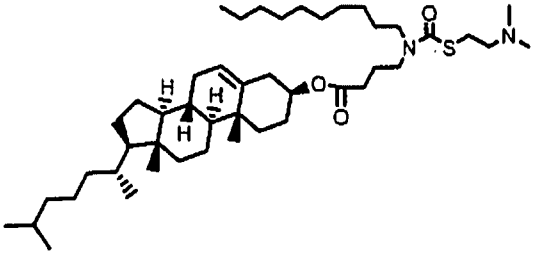

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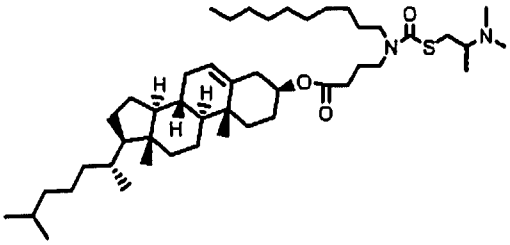

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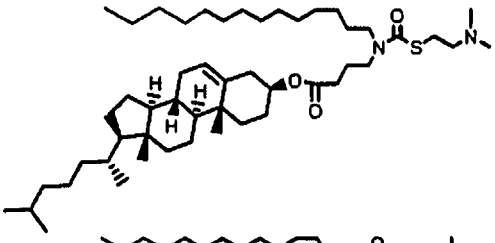

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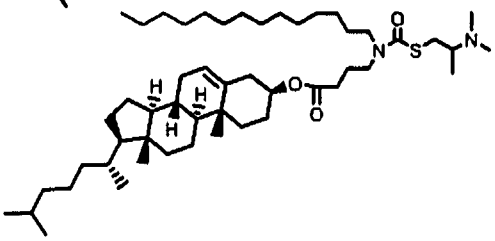

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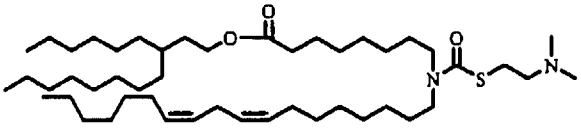

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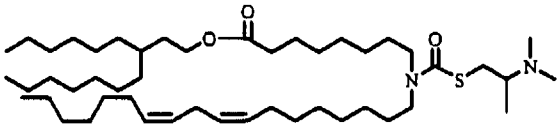

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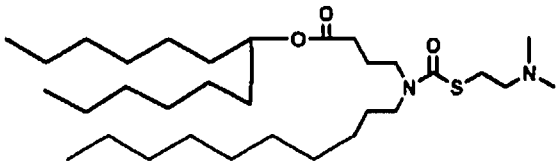

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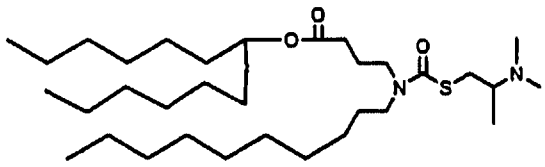


ATX-A-22

[0069] The compounds of formulas I, II, III, IV, and V may be a pharmaceutically acceptable salt thereof, in a lipid composition, comprising a nanoparticle or a bilayer of lipid molecules. The lipid bilayer preferably further comprises a neutral lipid or a polymer. The lipid composition preferably comprises a liquid medium. The composition preferably further encapsulates a nucleic acid. The nucleic acid preferably has an activity of suppressing the expression of the target gene by utilizing RNA interference (RNAi). The lipid composition preferably further comprises a nucleic acid and a neutral lipid or a polymer. The lipid composition preferably encapsulates the nucleic acid.

[0070] The compounds of formulas I, II, III, IV, and V form salts that are also within the scope of this disclosure. Reference to a compound of formulas I, II, III, IV, or V herein is understood to include reference to salts thereof, unless otherwise indicated. The term "salt(s)" as employed herein denotes acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. In addition, such salts of a compound of formulas I, II, III, IV, or V may contains a basic moiety, such as, but not limited to, a pyridine or imidazole, or an acidic moiety, such as, but not limited to, a carboxylic acid, and zwitterions ("inner salts"). The salts can be pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts, although other salts are also useful. Salts of the compounds of the formulas I, II, III, IV, or V may be formed, for example, by reacting a compound of formulas I, II, III, IV, or V with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0071] Exemplary acid addition salts include acetates, adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides, hydrobromides, hydroiodides, 2-hydroxyethanesulfonates, lactates, maleates, methanesulfonates, 2-napthalenesulfonates, nicotinates, nitrates, oxalates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates, sulfonates (such as those mentioned herein), tartarates, thiocyanates, toluenesulfonates (also known as tosylates) undecanoates, and the like. Additionally, acids which are generally considered suitable for the formation of pharmaceutically useful salts from basic pharmaceutical

compounds are discussed, for example, by S. Berge et al, *J. Pharmaceutical Sciences* (1977) 66(1)1-19; P. Gould, International *J. Pharmaceutics* (1986) 33 201-217; Anderson et al, The Practice of Medicinal Chemistry (1996), Academic Press, New York; and in The Orange Book (Food & Drug Administration, Washington, D.C. on their website). These disclosures are incorporated by reference herein.

[0072] Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glucamides, t-butyl amines, and salts with amino acids such as arginine or lysine. Basic nitrogen-containing groups may be quarternized with agents such as lower alkyl halides (*e.g.*, methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides), dialkyl sulfates (*e.g.*, dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (*e.g.*, decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides), arylalkyl halides (*e.g.*, benzyl and phenethyl bromides), and others.

[0073] All such acid and base salts are intended to be pharmaceutically acceptable salts within the scope of the disclosure and all acid and base salts are considered equivalent to the free forms of the corresponding compounds of formula I for purposes of the disclosure.

[0074] Compounds of formulas I, II, III, IV, and V can exist in unsolvated and solvated forms, including hydrated forms. In general, the solvated forms, with pharmaceutically acceptable solvents such as water, ethanol, and the like, are equivalent to the unsolvated forms for the purposes of this disclosure.

[0075] Compounds of formulas I, II, III, IV, and V and salts, solvates thereof, may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present disclosure.

[0076] Also within the scope of the present disclosure are polymorphs of the compounds of this disclosure (*i.e.*, polymorphs of the compounds of formula I are within the scope of this disclosure).

[0077] All stereoisomers (for example, geometric isomers, optical isomers, and the like) of the present compounds (including those of the salts, solvates, and prodrugs of the compounds as well as the salts and solvates of the prodrugs), such as those which may exist due to asymmetric carbons on various substituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons), rotameric forms, atropisomers, and diastereomeric forms, are contemplated within the scope of this disclosure. Individual stereoisomers of the

compounds of this disclosure may, for example, be substantially free of other isomers, or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the compounds herein can have the S or R configuration as defined by the IUPAC 1974 Recommendations. The use of the terms "salt", "solvate", and the like, is intended to equally apply to the salt and solvate of enantiomers, stereoisomers, rotamers, tautomers, racemates, or prodrugs of the disclosed compounds.

[0078] Classes of compounds that can be used as the chemotherapeutic agent (antineoplastic agent) include: alkylating agents, antimetabolites, natural products and their derivatives, hormones and steroids (including synthetic analogs), and synthetics. Examples of compounds within these classes are given below.

Lipid Particles

[0079] The description provides lipid particles comprising one or more therapeutic mRNA molecules encapsulated within the lipid particles.

[0080] In some embodiments, the mRNA is fully encapsulated within the lipid portion of the lipid particle such that the mRNA in the lipid particle is resistant in aqueous solution to nuclease degradation. In other embodiments, the lipid particles described herein are substantially non-toxic to mammals such as humans. The lipid particles typically have a mean diameter of from 30 nm to 150 nm, from 40 nm to 150 nm, from 50 nm to 150 nm, from 60 nm to 130 nm, from 70 nm to 110 nm, or from 70 to 90 nm. The lipid particles of the invention also typically have a lipid:RNA ratio (mass/mass ratio) of from 1:1 to 100:1, from 1:1 to 50:1, from 2:1 to 25:1, from 3:1 to 20:1, from 5:1 to 15:1, or from 5:1 to 10:1, or from 10:1 to 14:1, or from 9:1 to 20:1. In one embodiment, the lipid particles have a lipid: RNA ratio (mass/mass ratio) of 12:1. In another embodiment, the lipid particles have a lipid: mRNA ratio (mass/mass ratio) of 13:1.

[0081] In preferred embodiments, the lipid particles comprise an mRNA, a cationic lipid (*e.g.*, one or more cationic lipids or salts thereof described herein), a phospholipid, and a conjugated lipid that inhibits aggregation of the particles (*e.g.*, one or more PEG-lipid conjugates). The lipid particles can also include cholesterol. The lipid particles may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mRNA that express one or more polypeptides.

[0082] In the nucleic acid-lipid particles the mRNA may be fully encapsulated within the lipid portion of the particle, thereby protecting the nucleic acid from nuclease degradation. In preferred embodiments, a lipid particle comprising an mRNA is fully encapsulated within the lipid portion of the particle, thereby protecting the nucleic acid from nuclease degradation. In certain instances, the mRNA in the lipid particle is not substantially degraded after exposure of

the particle to a nuclease at 37°C for at least 20, 30, 45, or 60 minutes. In certain other instances, the mRNA in the lipid particle is not substantially degraded after incubation of the particle in serum at 37°C for at least 30, 45, or 60 minutes or at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 hours. In other embodiments, the mRNA is complexed with the lipid portion of the particle. One of the benefits of the formulations of the present invention is that the nucleic acid-lipid particle compositions are substantially non-toxic to mammals such as humans.

[0083] "Fully encapsulated" means that the nucleic acid (*e.g.*, mRNA) in the nucleic acid-lipid particle is not significantly degraded after exposure to serum or a nuclease assay that would significantly degrade free RNA. When fully encapsulated, preferably less than 25% of the nucleic acid in the particle is degraded in a treatment that would normally degrade 100% of free nucleic acid, more preferably less than 10%, and most preferably less than 5% of the nucleic acid in the particle is degraded. "Fully encapsulated" also means that the nucleic acid-lipid particles do not rapidly decompose into their component parts upon *in vivo* administration.

[0084] In the context of nucleic acids, full encapsulation may be determined by performing a membrane-impermeable fluorescent dye exclusion assay, which uses a dye that has enhanced fluorescence when associated with nucleic acid. Encapsulation is determined by adding the dye to a liposomal formulation, measuring the resulting fluorescence, and comparing it to the fluorescence observed upon addition of a small amount of nonionic detergent. Detergent-mediated disruption of the liposomal bilayer releases the encapsulated nucleic acid, allowing it to interact with the membrane-impermeable dye. Nucleic acid encapsulation may be calculated as $E = (I_0 - I)/I_0$, where/and I_0 refers to the fluorescence intensities before and after the addition of detergent.

[0085] In other embodiments, the present invention provides a nucleic acid-lipid particle composition comprising a plurality of nucleic acid-lipid particles.

[0086] The lipid particle comprises mRNA that is fully encapsulated within the lipid portion of the particles, such that from 30% to 100%, from 40% to 100%, from 50% to 100%, from 60% to 100%, from 70% to 100%, from 80% to 100%, from 90% to 100%, from 30% to 95%, from 40% to 95%, from 50% to 95%, from 60% to 95%, from 70% to 95%, from 80% to 95%, from 85% to 95%, from 90% to 95%, from 30% to 90%, from 40% to 90%, from 50% to 90%, from 60% to 90%, from 70% to 90%, from 80% to 90%, or at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% (or any fraction thereof or range therein) of the particles have the mRNA encapsulated therein.

[0087] Depending on the intended use of the lipid particles, the proportions of the components can be varied and the delivery efficiency of a particular formulation can be measured using assays know in the art.

Cationic lipids

[0088] The description includes synthesis of certain cationic lipid compounds. The compounds are particularly suitable for delivering polynucleotides to cells and tissues as demonstrated in subsequent sections. The lipomacrocycle compound described herein may be used for other purposes as well as, for example, recipients and additives.

[0089] The synthetic methods for the cationic lipid compounds can be synthesized with the skills in the art. The skilled of the art will recognize other methods to produce these compounds, and to produce also the other compounds of the description.

[0090] The cationic lipid compounds may be combined with an agent to form microparticles, nanoparticles, liposomes, or micelles. The agent to be delivered by the particles, liposomes, or micelles may be in the form of a gas, liquid, or solid, and the agent may be a polynucleotide, protein, peptide, or small molecule. The lipomacrocycle compounds may be combined with other cationic lipid compounds, polymers (synthetic or natural), surfactants, cholesterol, carbohydrates, proteins, or lipids, to form the particles. These particles may then optionally be combined with a pharmaceutical excipient to form a pharmaceutical composition.

[0091] The present description provides novel cationic lipid compounds and drug delivery systems based on the use of such cationic lipid compounds. The system may be used in the pharmaceutical/drug delivery arts to deliver polynucleotides, proteins, small molecules, peptides, antigen, or drugs, to a patient, tissue, organ, or cell. These novel compounds may also be used as materials for coating, additives, excipients, materials, or bioengineering.

[0092] The cationic lipid compounds of the present description provide for several different uses in the drug delivery art. The amine-containing portion of the cationic lipid compounds may be used to complex polynucleotides, thereby enhancing the delivery of polynucleotide and preventing their degradation. The cationic lipid compounds may also be used in the formation of picoparticles, nanoparticles, microparticles, liposomes, and micelles containing the agent to be delivered. Preferably, the cationic lipid compounds are biocompatible and biodegradable, and the formed particles are also biodegradable and biocompatible and may be used to provide controlled, sustained release of the agent to be delivered. These and their corresponding particles may also be responsive to pH changes given that these are protonated at

lower pH. They may also act as proton sponges in the delivery of an agent to a cell to cause endosome lysis.

[0093] In certain embodiments, the cationic lipid compounds are relatively non-cytotoxic. The cationic lipid compounds may be biocompatible and biodegradable. The cationic lipid may have a pK_a in the range of approximately 5.5 to approximately 7.5, more preferably between approximately 6.0 and approximately 7.0. It may be designed to have a desired pK_a between approximately 3.0 and approximately 9.0, or between approximately 5.0 and approximately 8.0. The cationic lipid compounds described herein are particularly attractive for drug delivery for several reasons: they contain amino groups for interacting with DNA, RNA, other polynucleotides, and other negatively charged agents, for buffering the pH, for causing endo-osmolysis, for protecting the agent to be delivered, they can be synthesized from commercially available starting materials; and/or they are pH responsive and can be engineered with a desired pK_a.

[0094] A composition containing a cationic lipid compound may be 30-70% cationic lipid compound, 0-60 % cholesterol, 0-30% phospholipid and 1-10% polyethylene glycol (PEG). Preferably, the composition is 30-40% cationic lipid compound, 40- 50% cholesterol, and 10-20% PEG. In other preferred embodiments, the composition is 50-75% cationic lipid compound, 20-40% cholesterol, and 5-10% phospholipid, and 1-10% PEG. The composition may contain 60-70% cationic lipid compound, 25-35% cholesterol, and 5-10% PEG. The composition may contain up to 90% cationic lipid compound and 2-15% helper lipid.

[0095] The formulation may be a lipid particle formulation, for example containing 8-30% compound, 5-30% helper lipid , and 0-20% cholesterol; 4-25% cationic lipid, 4-25% helper lipid, 2- 25% cholesterol, 10- 35% cholesterol-PEG, and 5% cholesterol-amine; or 2-30% cationic lipid, 2-30% helper lipid, 1- 15% cholesterol, 2- 35% cholesterol-PEG, and 1-20% cholesterol-amine; or up to 90% cationic lipid and 2-10% helper lipids, or even 100% cationic lipid.

Non-cationic Lipids

[0096] The non-cationic lipids that are used in lipid particles can be any of a variety of neutral uncharged, zwitterionic, or anionic lipids capable of producing a stable complex.

[0097] Non-limiting examples of non-cationic lipids include phospholipids such as lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC),

dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), palmitoyloleyol-phosphatidylglycerol (POPG), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane- 1 - carboxylate (DOPE-mal), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine, dimethyl-phosphatidylethanolamine, dielaidoyl-phosphatidylethanolamine (DEPE), stearoyloleoyl-phosphatidylethanolamine (SOPE), lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. Other diacylphosphatidylcholine and diacylphosphatidylethanolamine phospholipids can also be used. The acyl groups in these lipids are preferably acyl groups derived from fatty acids having C_{10}-C_{24} carbon chains, *e.g.*, lauroyl, myristoyl, palmitoyl, stearoyl, or oleoyl.

[0098] Additional examples of non-cationic lipids include sterols such as cholesterol and derivatives thereof. Non-limiting examples of cholesterol derivatives include polar analogues such as 5α-cholestanol, 5α-coprostanol, cholesteryl-(2'-hydroxy)-ethyl ether, cholesteryl-(4'- hydroxy)-butyl ether, and 6-ketocholestanol; non-polar analogues such as 5α-cholestane, cholestenone, 5α-cholestanone, 5α-cholestanone, and cholesteryl decanoate; and mixtures thereof. In preferred embodiments, the cholesterol derivative is a polar analogue such as cholesteryl-(4'-hydroxy)-butyl ether.

[0099] In some embodiments, the non-cationic lipid present in lipid particles comprises or consists of a mixture of one or more phospholipids and cholesterol or a derivative thereof. In other embodiments, the non-cationic lipid present in the lipid particles comprises or consists of one or more phospholipids, *e.g.*, a cholesterol-free lipid particle formulation. In yet other embodiments, the non-cationic lipid present in the lipid particles comprises or consists of cholesterol or a derivative thereof, *e.g.*, a phospholipid-free lipid particle formulation.

[0100] Other examples of non-cationic lipids include nonphosphorous containing lipids such as, *e.g.*, stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerolricinoleate, hexadecyl stereate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethyloxylated fatty acid amides, dioctadecyldimethyl ammonium bromide, ceramide, and sphingomyelin.

[0101] In some embodiments, the non-cationic lipid comprises from 10 mol % to 60 mol %, from 20 mol % to 55 mol %, from 20 mol % to 45 mol %, 20 mol % to 40 mol %, from 25 mol % to 50 mol %, from 25 mol % to 45 mol %, from 30 mol % to 50 mol %, from 30 mol % to 45 mol %, from 30 mol % to 40 mol %, from 35 mol % to 45 mol %, from 37 mol % to 42

mol %, or 35 mol %, 36 mol %, 37 mol %, 38 mol %, 39 mol %, 40 mol %, 41 mol %, 42 mol %, 43 mol %, 44 mol %, or 45 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0102] In embodiments where the lipid particles contain a mixture of phospholipid and cholesterol or a cholesterol derivative, the mixture may comprise up to 40 mol %, 45 mol %, 50 mol %, 55 mol %, or 60 mol % of the total lipid present in the particle.

[0103] In some embodiments, the phospholipid component in the mixture may comprise from 2 mol % to 20 mol %, from 2 mol % to 15 mol %, from 2 mol % to 12 mol %, from 4 mol % to 15 mol %, or from 4 mol % to 10 mol % (or any fraction thereof or range therein) of the total lipid present in the particle. In certain preferred embodiments, the phospholipid component in the mixture comprises from 5 mol % to 10 mol %, from 5 mol % to 9 mol %, from 5 mol % to 8 mol %, from 6 mol % to 9 mol %, from 6 mol % to 8 mol %, or 5 mol %, 6 mol %, 7 mol %, 8 mol %, 9 mol %, or 10 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0104] In other embodiments, the cholesterol component in the mixture may comprise from 25 mol % to 45 mol %, from 25 mol % to 40 mol %, from 30 mol % to 45 mol %, from 30 mol % to 40 mol %, from 27 mol % to 37 mol %, from 25 mol % to 30 mol %, or from 35 mol % to 40 mol % (or any fraction thereof or range therein) of the total lipid present in the particle. In certain preferred embodiments, the cholesterol component in the mixture comprises from 25 mol % to 35 mol %, from 27 mol % to 35 mol %, from 29 mol % to 35 mol %, from 30 mol % to 35 mol %, from 30 mol % to 34 mol %, from 31 mol % to 33 mol %, or 30 mol %, 31 mol %, 32 mol %, 33 mol %, 34 mol %, or 35 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0105] In embodiments where the lipid particles are phospholipid-free, the cholesterol or derivative thereof may comprise up to 25 mol %, 30 mol %, 35 mol %, 40 mol %, 45 mol %, 50 mol %, 55 mol %, or 60 mol % of the total lipid present in the particle.

[0106] In some embodiments, the cholesterol or derivative thereof in the phospholipid-free lipid particle formulation may comprise from 25 mol % to 45 mol %, from 25 mol % to 40 mol %, from 30 mol % to 45 mol %, from 30 mol % to 40 mol %, from 31 mol % to 39 mol %, from 32 mol % to 38 mol %, from 33 mol % to 37 mol %, from 35 mol % to 45 mol %, from 30 mol % to 35 mol %, from 35 mol % to 40 mol %, or 30 mol %, 31 mol %, 32 mol %, 33 mol %, 34 mol %, 35 mol %, 36 mol %, 37 mol %, 38 mol %, 39 mol %, or 40 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0107] In other embodiments, the non-cationic lipid comprises from 5 mol % to 90 mol %, from 10 mol % to 85 mol %, from 20 mol % to 80 mol %, 10 mol % (*e.g.*, phospholipid only), or 60 mol % (*e.g.*, phospholipid and cholesterol or derivative thereof) (or any fraction thereof or range therein) of the total lipid present in the particle.

[0108] The percentage of non-cationic lipid present in the lipid particles is a target amount, and that the actual amount of non-cationic lipid present in the formulation may vary, for example, by ± 5 mol %.

Lipid Conjugates

[0109] In addition to cationic, the lipid particles described herein may further comprise a lipid conjugate. The conjugated lipid is useful in that it prevents the aggregation of particles. Suitable conjugated lipids include, but are not limited to, PEG-lipid conjugates, cationic-polymer- lipid conjugates, and mixtures thereof.

[0110] In a preferred embodiment, the lipid conjugate is a PEG-lipid. Examples of PEG- lipids include, but are not limited to, PEG coupled to dialkyloxypropyls (PEG-DAA), PEG coupled to diacylglycerol (PEG-DAG), PEG coupled to phospholipids such as phosphatidylethanolamine (PEG-PE), PEG conjugated to ceramides, PEG conjugated to cholesterol or a derivative thereof, and mixtures thereof.

[0111] PEG is a linear, water-soluble polymer of ethylene PEG repeating units with two terminal hydroxyl groups. PEGs are classified by their molecular weights; and include the following: monomethoxypolyethylene glycol (MePEG-OH), monomethoxypolyethylene glycol-succinate (MePEG-S), monomethoxypolyethylene glycol-succinimidyl succinate (MePEG-S-NHS), monomethoxypolyethylene glycol-amine (MePEG-NH_2), monomethoxypolyethylene glycol-tresylate (MePEG-TRES), monomethoxypolyethylene glycol-imidazolyl-carbonyl (MePEG-IM), as well as such compounds containing a terminal hydroxyl group instead of a terminal methoxy group (*e.g.*, HO-PEG-S, HO-PEG-S-NHS, HO-PEG-NH_2).

[0112] The PEG moiety of the PEG-lipid conjugates described herein may comprise an average molecular weight ranging from 550 daltons to 10,000 daltons. In certain instances, the PEG moiety has an average molecular weight of from 750 daltons to 5,000 daltons (*e.g.*, from 1,000 daltons to 5,000 daltons, from 1,500 daltons to 3,000 daltons, from 750 daltons to 3,000 daltons, from 750 daltons to 2,000 daltons). In preferred embodiments, the PEG moiety has an average molecular weight of 2,000 daltons or 750 daltons.

[0113] In certain instances, the PEG can be optionally substituted by an alkyl, alkoxy, acyl, or aryl group. The PEG can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG to a lipid can be used

including, *e.g.*, non-ester-containing linker moieties and ester-containing linker moieties. In a preferred embodiment, the linker moiety is a non-ester-containing linker moiety. Suitable non-ester-containing linker moieties include, but are not limited to, amido (-C(O)NH-), amino (-NR-), carbonyl (-C(O)-), carbamate (-NHC(O)O-), urea (-NHC(O)NH-), disulphide (-S-S-), ether (-O-), succinyl (-(O)CCH_2CH_2C(O)-), succinamidyl (-NHC(O)CH_2CH_2C(O)NH-), ether, disulphide, as well as combinations thereof (such as a linker containing both a carbamate linker moiety and an amido linker moiety). In a preferred embodiment, a carbamate linker is used to couple the PEG to the lipid.

[0114] In other embodiments, an ester-containing linker moiety is used to couple the PEG to the lipid. Suitable ester-containing linker moieties include, *e.g.*, carbonate (-OC(O)O-), succinoyl, phosphate esters (-O-(O)POH-O-), sulfonate esters, and combinations thereof.

[0115] Phosphatidylethanolamines having a variety of acyl chain groups of varying chain lengths and degrees of saturation can be conjugated to PEG to form the lipid conjugate. Such phosphatidylethanolamines are commercially available, or can be isolated or synthesized using conventional techniques known to those of skill in the art. Phosphatidylethanolamines containing saturated or unsaturated fatty acids with carbon chain lengths in the range of C_{10} to C_{20} are preferred. Phosphatidylethanolamines with mono- or di-unsaturated fatty acids and mixtures of saturated and unsaturated fatty acids can also be used. Suitable phosphatidylethanolamines include, but are not limited to, dimyristoyl-phosphatidylethanolamine (DMPE), dipalmitoyl-phosphatidylethanolamine (DPPE), dioleoyl-phosphatidylethanolamine (DOPE), and distearoyl-phosphatidylethanolamine (DSPE).

[0116] The term "diacylglycerol" or "DAG" includes a compound having 2 fatty acyl chains, R^1 and R^2, both of which have independently between 2 and 30 carbons bonded to the 1- and 2-position of glycerol by ester linkages. The acyl groups can be saturated or have varying degrees of unsaturation. Suitable acyl groups include, but are not limited to, lauroyl (C_{12}), myristoyl (C_{14}), palmitoyl (C_{16}), stearoyl (C_{18}), and icosoyl (C_{20}). In preferred embodiments, R^1 and R^2 are the same, *i.e.*, R^1 and R^2 are both myristoyl (*i.e.*, dimyristoyl), R^1 and R^2 are both stearoyl (*i.e.*, distearoyl).

[0117] The term "dialkyloxypropyl" or "DAA" includes a compound having 2 alkyl chains, R and R , both of which have independently between 2 and 30 carbons. The alkyl groups can be saturated or have varying degrees of unsaturation.

[0118] Preferably, the PEG-DAA conjugate is a PEG-didecyloxypropyl (C_{10}) conjugate, a PEG-dilauryloxypropyl (C_{12}) conjugate, a PEG-dimyristyloxypropyl (C_{14}) conjugate, a PEG-dipalmityloxypropyl (C_{16}) conjugate, or a PEG-distearyloxypropyl (C_{18})

conjugate. In these embodiments, the PEG preferably has an average molecular weight of 750 or 2,000 daltons. In particular embodiments, the terminal hydroxyl group of the PEG is substituted with a methyl group.

[0119] In addition to the foregoing, other hydrophilic polymers can be used in place of PEG. Examples of suitable polymers that can be used in place of PEG include, but are not limited to, polyvinylpyrrolidone, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide and polydimethylacrylamide, polylactic acid, polyglycolic acid, and derivatized celluloses such as hydroxymethylcellulose or hydroxyethylcellulose.

[0120] In some embodiments, the lipid conjugate (*e.g.*, PEG-lipid) comprises from 0.1 mol % to 2 mol %, from 0.5 mol % to 2 mol %, from 1 mol % to 2 mol %, from 0.6 mol % to 1.9 mol %, from 0.7 mol % to 1.8 mol %, from 0.8 mol % to 1.7 mol %, from 0.9 mol % to 1.6 mol %, from 0.9 mol % to 1.8 mol %, from 1 mol % to 1.8 mol %, from 1 mol % to 1.7 mol %, from 1.2 mol % to 1.8 mol %, from 1.2 mol % to 1.7 mol %, from 1.3 mol % to 1.6 mol %, or from 1.4 mol % to 1.5 mol % (or any fraction thereof or range therein) of the total lipid present in the particle. In other embodiments, the lipid conjugate (*e.g.*, PEG-lipid) comprises from 0 mol % to 20 mol %, from 0.5 mol % to 20 mol %, from 2 mol % to 20 mol %, from 1.5 mol % to 18 mol %, from 2 mol % to 15 mol %, from 4 mol % to 15 mol %, from 2 mol % to 12 mol %, from 5 mol % to 12 mol %, or 2 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0121] In further embodiments, the lipid conjugate (*e.g.*, PEG-lipid) comprises from 4 mol % to 10 mol %, from 5 mol % to 10 mol %, from 5 mol % to 9 mol %, from 5 mol % to 8 mol %, from 6 mol % to 9 mol %, from 6 mol % to 8 mol %, or 5 mol %, 6 mol %, 7 mol %, 8 mol %, 9 mol %, or 10 mol % (or any fraction thereof or range therein) of the total lipid present in the particle.

[0122] The percentage of lipid conjugate (*e.g.*, PEG-lipid) present in the lipid particles of the invention is a target amount, and the actual amount of lipid conjugate present in the formulation may vary, for example, by ± 2 mol %. One of ordinary skill in the art will appreciate that the concentration of the lipid conjugate can be varied depending on the lipid conjugate employed and the rate at which the lipid particle is to become fusogenic.

[0123] By controlling the composition and concentration of the lipid conjugate, one can control the rate at which the lipid conjugate exchanges out of the lipid particle and, in turn, the rate at which the lipid particle becomes fusogenic. In addition, other variables including, *e.g.*, pH, temperature, or ionic strength, can be used to vary and/or control the rate at which the lipid particle becomes fusogenic. Other methods which can be used to control the rate at which the

lipid particle becomes fusogenic will become apparent to those of skill in the art upon reading this disclosure. Also, by controlling the composition and concentration of the lipid conjugate, one can control the lipid particle size.

Compositions and Formulations for Administration

[0124] The nucleic acid-lipid compositions of this disclosure may be administered by various routes, for example, to effect systemic delivery via intravenous, parenteral, intraperitoneal, or topical routes. In some embodiments, a siRNA may be delivered intracellularly, for example, in cells of a target tissue such as lung or liver, or in inflamed tissues. In some embodiments, this disclosure provides a method for delivery of siRNA *in vivo*. A nucleic acid-lipid composition may be administered intravenously, subcutaneously, or intraperitoneally to a subject. In some embodiments, the disclosure provides methods for *in vivo* delivery of interfering RNA to the lung of a mammalian subject.

[0125] In some embodiments, this disclosure provides a method of treating a disease or disorder in a mammalian subject. A therapeutically effective amount of a composition of this disclosure containing a nucleic, a cationic lipid, an amphiphile, a phospholipid, cholesterol, and a PEG-linked cholesterol may be administered to a subject having a disease or disorder associated with expression or overexpression of a gene that can be reduced, decreased, downregulated, or silenced by the composition.

[0126] The compositions and methods of the disclosure may be administered to subjects by a variety of mucosal administration modes, including by oral, rectal, vaginal, intranasal, intrapulmonary, or transdermal or dermal delivery, or by topical delivery to the eyes, ears, skin, or other mucosal surfaces. In some aspects of this disclosure, the mucosal tissue layer includes an epithelial cell layer. The epithelial cell can be pulmonary, tracheal, bronchial, alveolar, nasal, buccal, epidermal, or gastrointestinal. Compositions of this disclosure can be administered using conventional actuators such as mechanical spray devices, as well as pressurized, electrically activated, or other types of actuators.

[0127] Compositions of this disclosure may be administered in an aqueous solution as a nasal or pulmonary spray and may be dispensed in spray form by a variety of methods known to those skilled in the art. Pulmonary delivery of a composition of this disclosure is achieved by administering the composition in the form of drops, particles, or spray, which can be, for example, aerosolized, atomized, or nebulized. Particles of the composition, spray, or aerosol can be in either a liquid or solid form. Preferred systems for dispensing liquids as a nasal spray are disclosed in U.S. Pat. No. 4,511,069. Such formulations may be conveniently prepared by

dissolving compositions according to the present disclosure in water to produce an aqueous solution, and rendering said solution sterile. The formulations may be presented in multi-dose containers, for example in the sealed dispensing system disclosed in U.S. Pat. No. 4,511,069. Other suitable nasal spray delivery systems have been described in Transdermal Systemic Medication, Y. W. Chien ed., Elsevier Publishers, New York, 1985; and in U.S. Pat. No. 4,778,810. Additional aerosol delivery forms may include, *e.g.*, compressed air-, jet-, ultrasonic-, and piezoelectric nebulizers, which deliver the biologically active agent dissolved or suspended in a pharmaceutical solvent, *e.g.*, water, ethanol, or mixtures thereof.

[0128] Nasal and pulmonary spray solutions of the present disclosure typically comprise the drug or drug to be delivered, optionally formulated with a surface active agent, such as a nonionic surfactant (*e.g.*, polysorbate-80), and one or more buffers. In some embodiments of the present disclosure, the nasal spray solution further comprises a propellant. The pH of the nasal spray solution may be from pH 6.8 to 7.2. The pharmaceutical solvents employed can also be a slightly acidic aqueous buffer of pH 4-6. Other components may be added to enhance or maintain chemical stability, including preservatives, surfactants, dispersants, or gases.

[0129] In some embodiments, this disclosure is a pharmaceutical product which includes a solution containing a composition of this disclosure and an actuator for a pulmonary, mucosal, or intranasal spray or aerosol.

[0130] A dosage form of the composition of this disclosure can be liquid, in the form of droplets or an emulsion, or in the form of an aerosol.

[0131] A dosage form of the composition of this disclosure can be solid, which can be reconstituted in a liquid prior to administration. The solid can be administered as a powder. The solid can be in the form of a capsule, tablet, or gel.

[0132] To formulate compositions for pulmonary delivery within the present disclosure, the biologically active agent can be combined with various pharmaceutically acceptable additives, as well as a base or carrier for dispersion of the active agent(s). Examples of additives include pH control agents such as arginine, sodium hydroxide, glycine, hydrochloric acid, citric acid, and mixtures thereof. Other additives include local anesthetics (*e.g.*, benzyl alcohol), isotonizing agents (*e.g.*, sodium chloride, mannitol, sorbitol), adsorption inhibitors (*e.g.*, Tween 80), solubility enhancing agents (*e.g.*, cyclodextrins and derivatives thereof), stabilizers (*e.g.*, serum albumin), and reducing agents (*e.g.*, glutathione). When the composition for mucosal delivery is a liquid, the tonicity of the formulation, as measured with reference to the tonicity of 0.9% (w/v) physiological saline solution taken as unity, is typically adjusted to a value at which

no substantial, irreversible tissue damage will be induced in the mucosa at the site of administration. Generally, the tonicity of the solution is adjusted to a value of 1/3 to 3, more typically 1/2 to 2, and most often 3/4 to 1.7.

[0133] The biologically active agent may be dispersed in a base or vehicle, which may comprise a hydrophilic compound having a capacity to disperse the active agent and any desired additives. The base may be selected from a wide range of suitable carriers, including but not limited to, copolymers of polycarboxylic acids or salts thereof, carboxylic anhydrides (*e.g.*, maleic anhydride) with other monomers (*e.g.*, methyl(meth)acrylate, acrylic acid, etc.), hydrophilic vinyl polymers such as polyvinyl acetate, polyvinyl alcohol, polyvinylpyrrolidone, cellulose derivatives such as hydroxymethylcellulose, hydroxypropylcellulose, etc., and natural polymers such as chitosan, collagen, sodium alginate, gelatin, hyaluronic acid, and nontoxic metal salts thereof. Often, a biodegradable polymer is selected as a base or carrier, for example, polylactic acid, poly(lactic acid-glycolic acid) copolymer, polyhydroxybutyric acid, poly(hydroxybutyric acid-glycolic acid) copolymer, and mixtures thereof. Alternatively or additionally, synthetic fatty acid esters such as polyglycerin fatty acid esters, sucrose fatty acid esters, etc., can be employed as carriers. Hydrophilic polymers and other carriers can be used alone or in combination, and enhanced structural integrity can be imparted to the carrier by partial crystallization, ionic bonding, crosslinking, and the like. The carrier can be provided in a variety of forms, including fluid or viscous solutions, gels, pastes, powders, microspheres, and films for direct application to the nasal mucosa. The use of a selected carrier in this context may result in promotion of absorption of the biologically active agent.

[0134] Formulations for mucosal, nasal, or pulmonary delivery may contain a hydrophilic low molecular weight compound as a base or excipient. Such hydrophilic low molecular weight compounds provide a passage medium through which a water-soluble active agent, such as a physiologically active peptide or protein, may diffuse through the base to the body surface where the active agent is absorbed. The hydrophilic low molecular weight compound optionally absorbs moisture from the mucosa or the administration atmosphere and dissolves the water-soluble active peptide. The molecular weight of the hydrophilic low molecular weight compound is generally not more than 10,000 and preferably not more than 3,000. Examples of hydrophilic low molecular weight compounds include polyol compounds, such as oligo-, di- and monosaccarides including sucrose, mannitol, lactose, L-arabinose, D-erythrose, D-ribose, D-xylose, D-mannose, D-galactose, lactulose, cellobiose, gentibiose, glycerin, polyethylene glycol, and mixtures thereof. Further examples of hydrophilic low

molecular weight compounds include N-methylpyrrolidone, alcohols (*e.g.*, oligovinyl alcohol, ethanol, ethylene glycol, propylene glycol, *etc.*), and mixtures thereof.

[0135] The compositions of this disclosure may alternatively contain as pharmaceutically acceptable carriers substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, and wetting agents, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, and mixtures thereof. For solid compositions, conventional nontoxic pharmaceutically acceptable carriers can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

[0136] In certain embodiments of the disclosure, the biologically active agent may be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active agent can be prepared with carriers that will protect against rapid release, for example a controlled release vehicle such as a polymer, microencapsulated delivery system, or bioadhesive gel. Prolonged delivery of the active agent, in various compositions of the disclosure can be brought about by including in the composition agents that delay absorption, for example, aluminum monosterate hydrogels and gelatin.

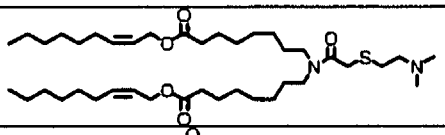
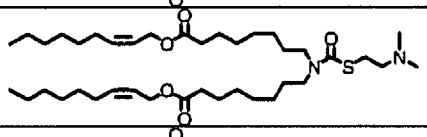
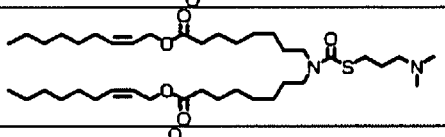
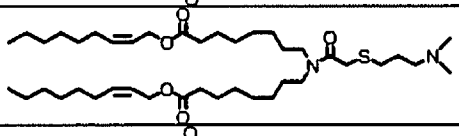
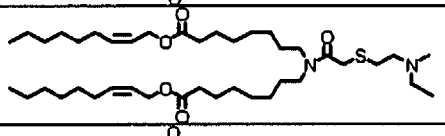
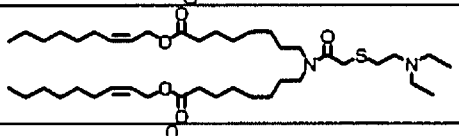
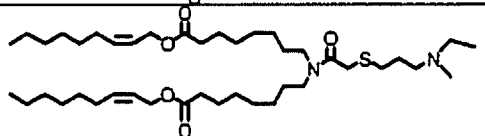
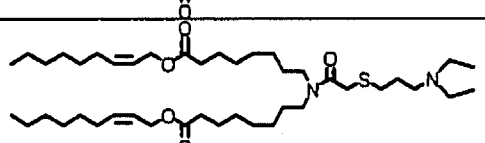
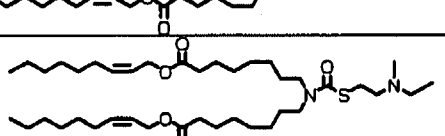
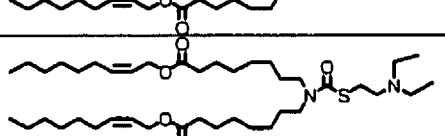
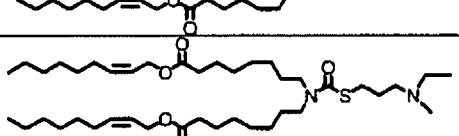
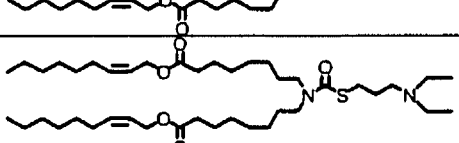
[0137] While this disclosure has been described in relation to certain embodiments, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that this disclosure includes additional embodiments, and that some of the details described herein may be varied considerably without departing from this disclosure. This disclosure includes such additional embodiments, modifications, and equivalents. In particular, this disclosure includes any combination of the features, terms, or elements of the various illustrative components and examples.

Examples

Example 1.

[0138] Exemplary compounds of formula I are provided in Table 1.

Table 1

Lipid ID	Novel Lipid	MW	pKa	KD @ 0.3 mg/kg
ATX-001		695.1	8.9	~0
ATX-002		681	8.7	98
ATX-003		695.1	9.3	~0
ATX-004		709.13	9.4	~0
ATX-005		709.13	9.0	~0
ATX-006		723.15	9.8	~0
ATX-007		723.15	9.5	n/a
ATX-008		737.18	10.3	n/a
ATX-009		695.1	8.8	~0
ATX-010		709.13	9.6	30
ATX-011		709.13	9.4	n/a
ATX-012		723.15	10.2	~0

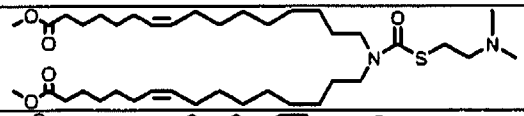
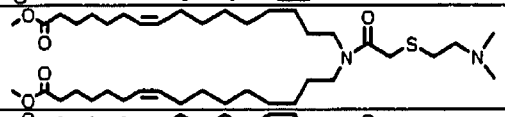
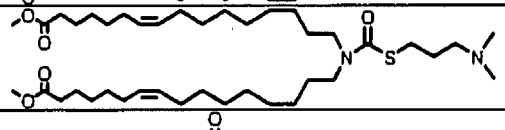
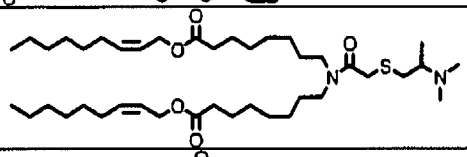
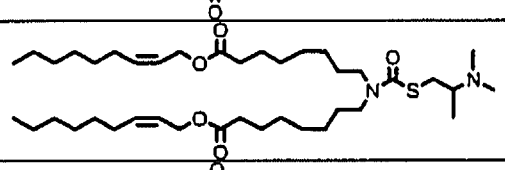
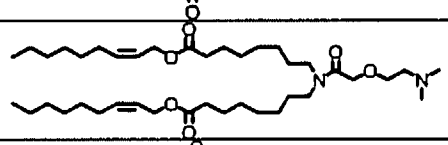
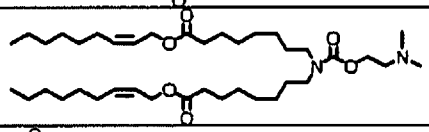
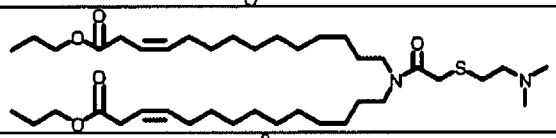
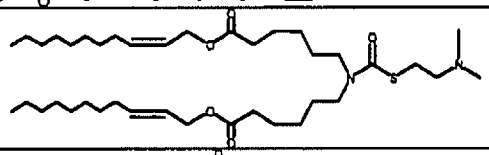
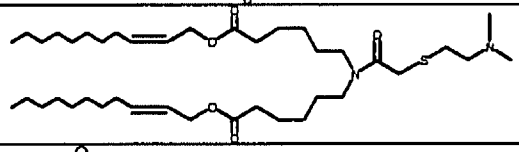
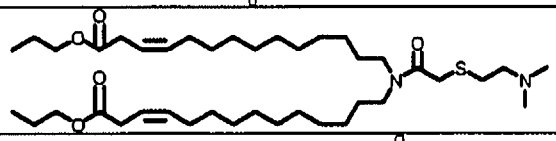
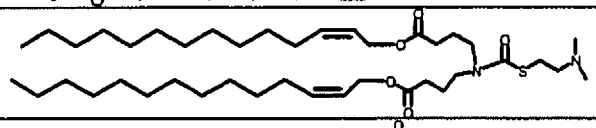
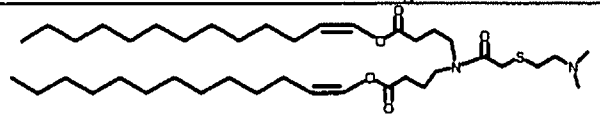
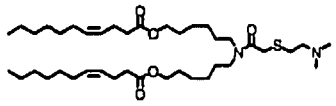
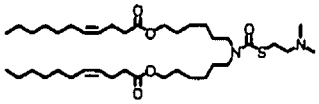
ATX-013		681.01		n/a
ATX-014		695.1		n/a
ATX-015		695.1		n/a
ATX-016		709.13		15
ATX-017		695.1		n/a
ATX-021		679.04		n/a
ATX-022		665.01		n/a
ATX-023		695.1		n/a
ATX-026		681.07		n/a
ATX-027		695.1		n/a
ATX-028		681.07		n/a
ATX-029		681.1		n/a
ATX-030		695.1		n/a

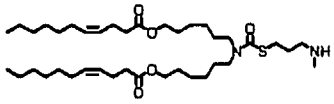
Table 1 shows the name and structure of each compound, its molecular weight, its pKa, and its knockdown bioactivity (KD) in an assay described below in Example 19.

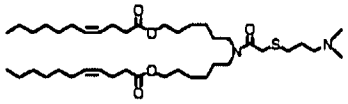
[0139] Exemplary compounds of formulas II and III are provided in Tables 2, and 3.

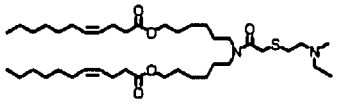
Table 2

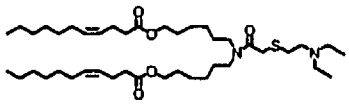
1) 

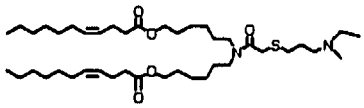
2) 

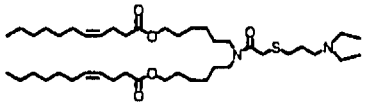
3) 

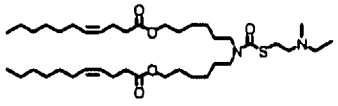
4) 

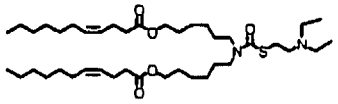
5) 

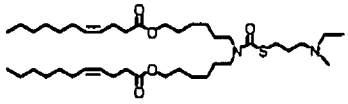
6) 

7) 

8) 

9) 

10) 

11) 

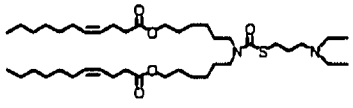
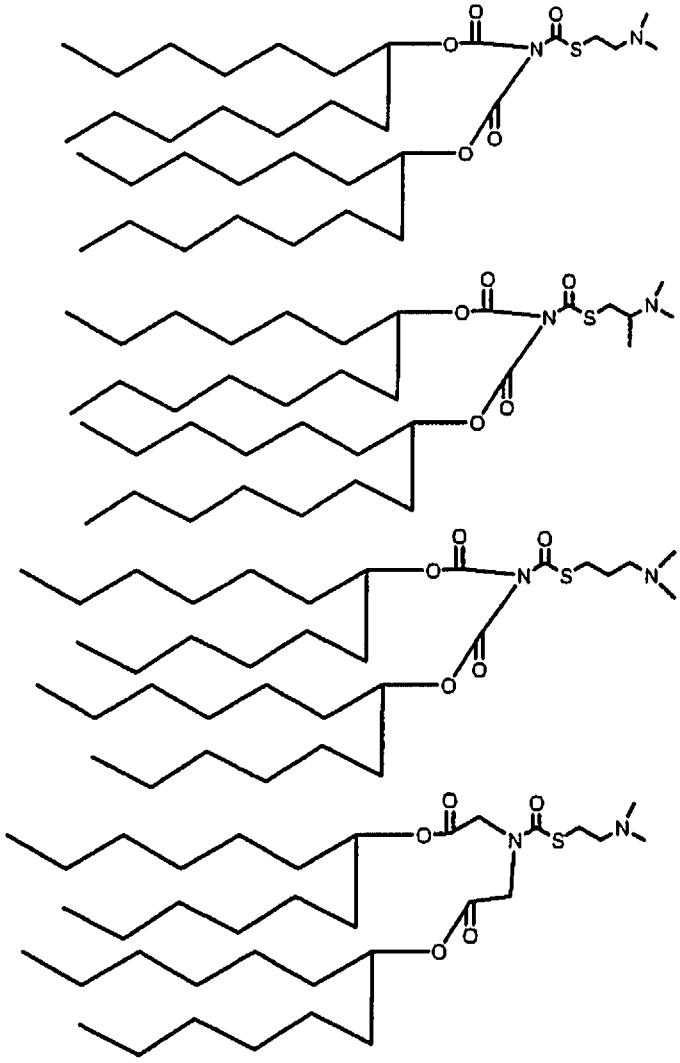
12) 

Table 3

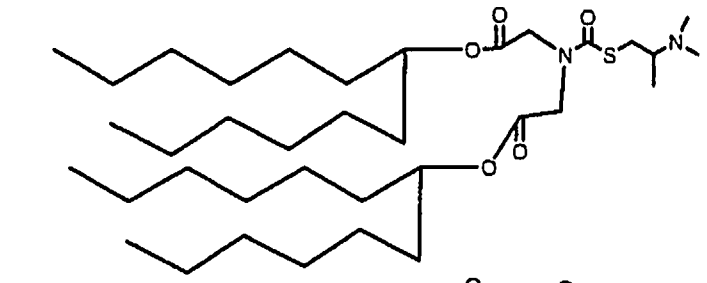


ATX-B-1

ATX-B-2

ATX-B-3

ATX-B-4



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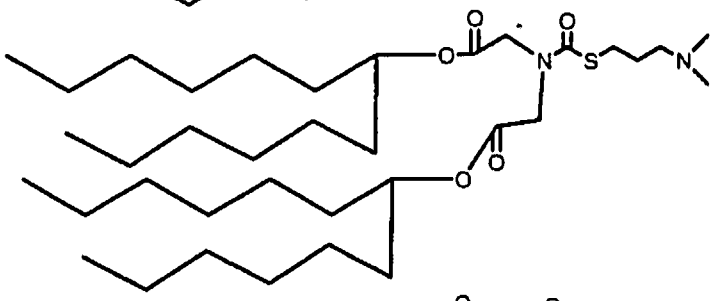

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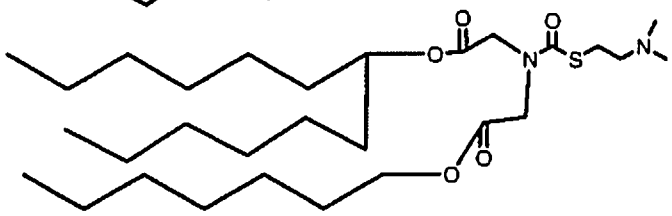
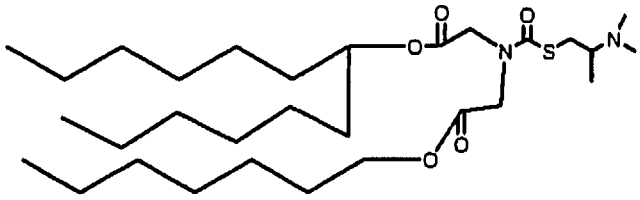
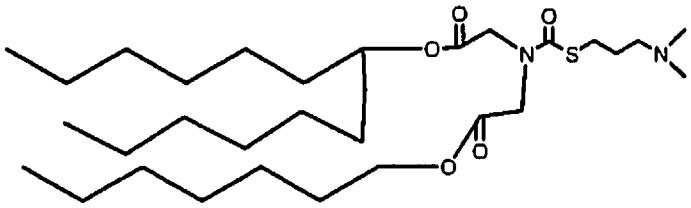


ATX-B-7



ATX-B-8



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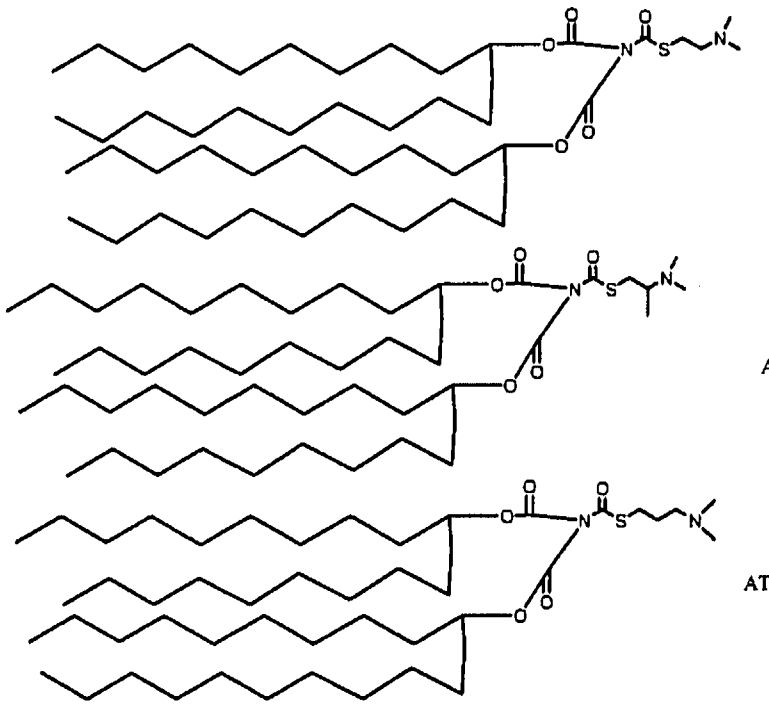
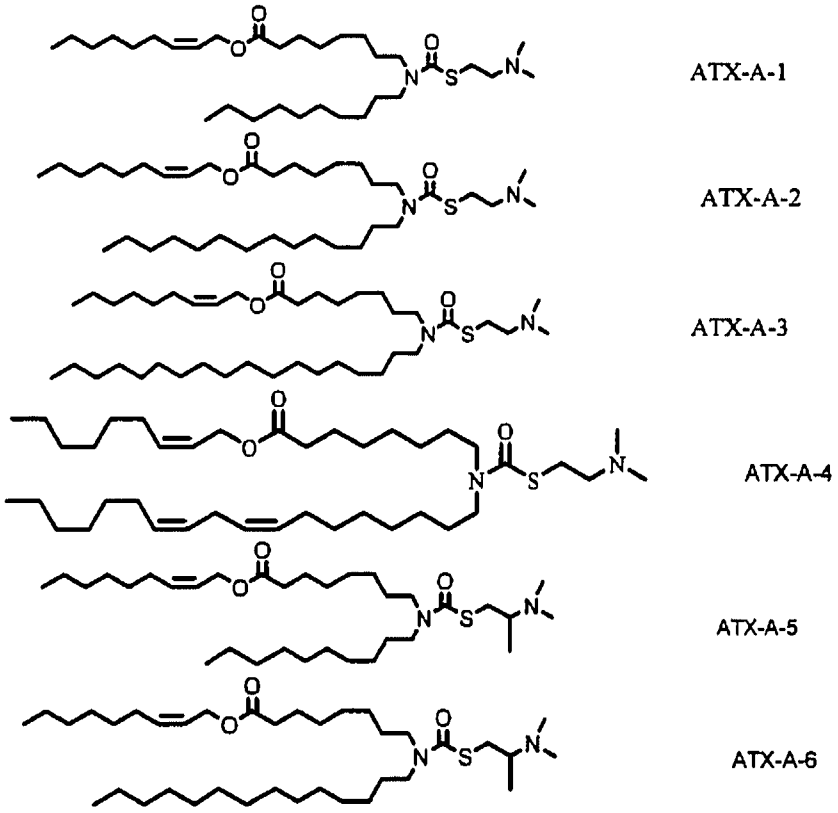
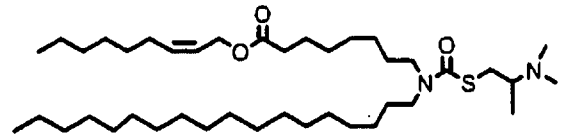


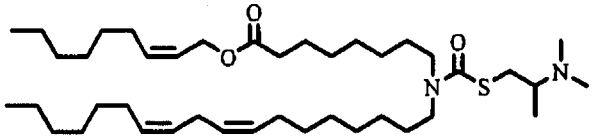
[0140] Exemplary compounds of formula V are provided in Table 4.

Table 4





ATX-A-7



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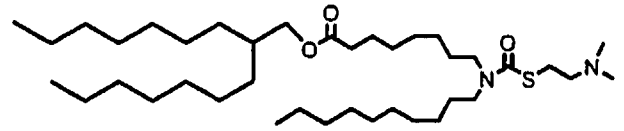

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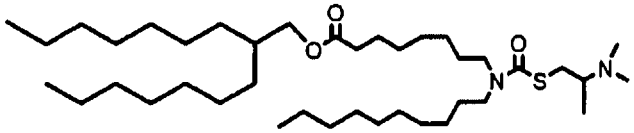

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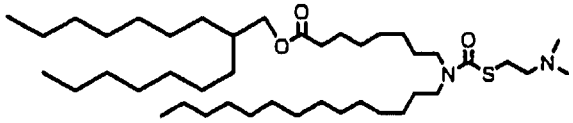

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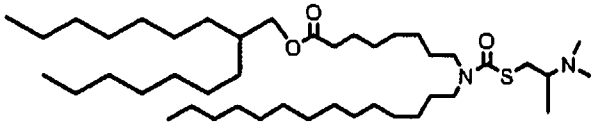
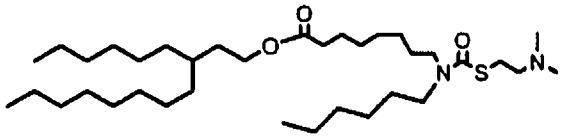


ATX-A-12



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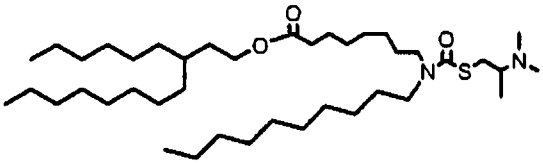

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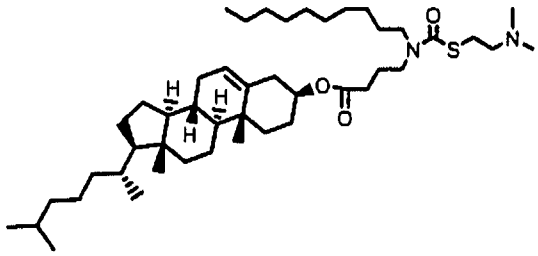
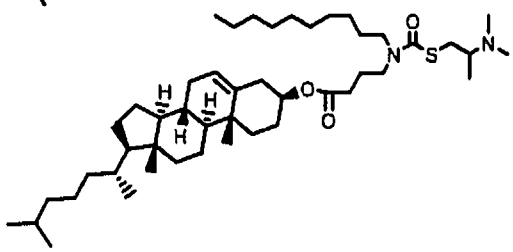


ATX-A-15



ATX-A-16

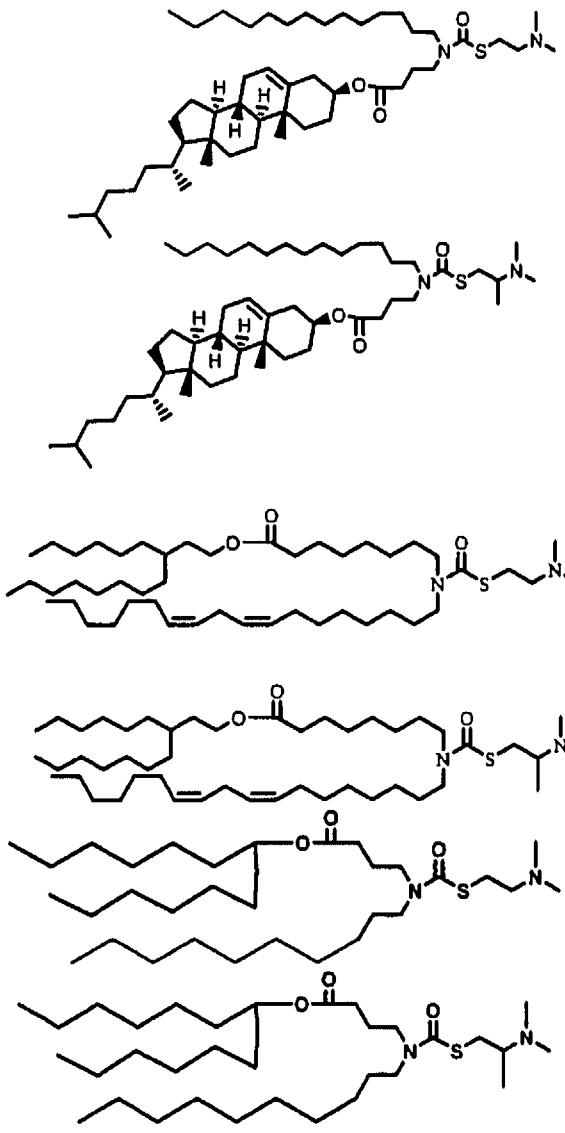
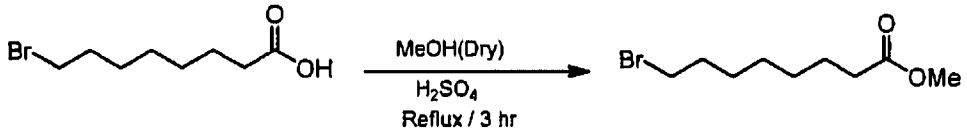


Table 1 shows the name and structure of each compound, its molecular weight, its pKa, and its knockdown bioactivity (KD) in an assay described below in Example 19.

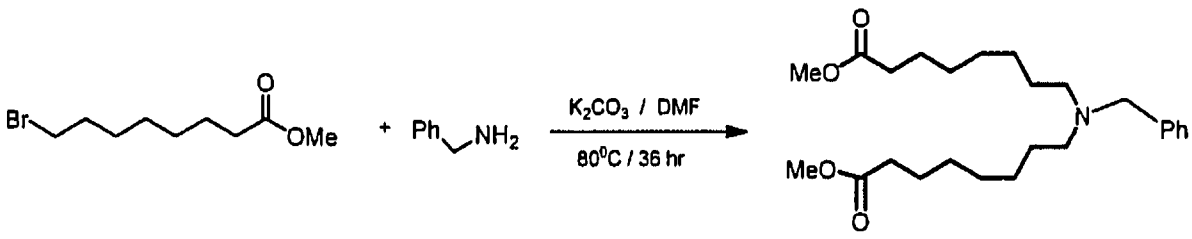
Example 2. Synthesis of methyl 8-bromooctanoate



[0141] Under N2 atmosphere, 8-bromooctanoic acid was dissolved in dry methanol. Concentrated H_2SO_4 was added drop-wise and the reaction mixture was stirred under reflux for three hours.

[0142] The reaction was monitored by thin layer chromatography until completed. Solvent was completely removed under vacuum. The reaction mixture was diluted with ethyl acetate and washed with water. The water layer was re-extracted with ethyl acetate. The total organic layer was washed with a saturated $NaHCO_3$ solution. The organic layer was washed again with water and finally washed with brine. The product was dried over anhydrous Na_2SO_4 and concentrated.

Example 3. Synthesis of dimethyl 8,8'-(benzanediyl)dioctanoate



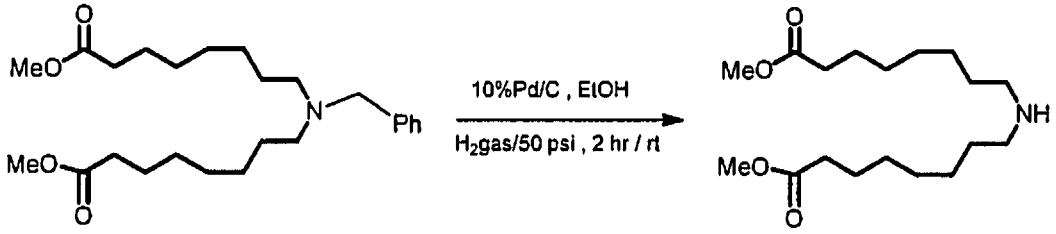
[0143] Dry K_2CO_3 was taken and added to dry dimethylformamide under N_2. Benzyl amine in dimethylformamide was slowly added. Methyl 8-bromooctanoate dissolved in dimethylformamide was then added at room temperature. The reaction mixture was heated to 80° C and the reaction was maintained for 36 hours with stirring.

[0144] The reaction was monitored by thin layer chromatography until completed. The reaction product was cooled to room temperature and water was added. The compound was extracted with ethyl acetate. The water layer was re-extracted with ethyl acetate. The total organic layer was washed with water and finally with brine solution. The product was dried over anhydrous Na_2SO_4 and concentrated.

[0145] The reaction product was purified by silica gel column chromatography in 3% methanol in chloroform 44 gm of pure product was recovered.

[0146] Using TLC system of 10% methanol in chloroform, the product migrated with a Rf: 0.8, visualizing by charring in ninhydrine. The overall yield was 82%. The compound was a light brown liquid. The structure was confirmed by ^{1}H-NMR.

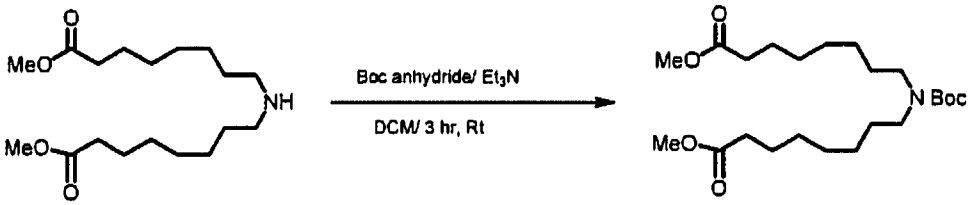
Example 4. Synthesis of dimethyl 8,8'-azanediyldioctanoate



[0147] Dimethyl 8,8'-(benzanediyl)dioctanoate was transferred to hydrogenation glass vessel, and ethanol was added followed by 10% Pd/C. The reaction mixture was shaken in a Parr-shaker apparatus under 50 pounds per square inch [psi] H_2 atmosphere pressure for two hours at room temperature.

[0148] The reaction product was filtered through celite and washed with hot ethyl acetate. The filtrate was concentrated under vacuum.

Example 5. Synthesis of dimethyl 8,8'-((tertbutoxycarbonyl)azanedil) dioctanoate



[0149] Dimethyl 8,8'-azanediyldioctanoate was transferred to DCM and Et_3N to the reaction mass and cooled to 0°C. Boc anhydride diluted in DCM was added drop to the above reaction. After the addition was completed, the reaction mixture was stirred at room temperature for three hours.

[0150] The reaction was quenched with water and the DCM layer was separated. The water phase was re-extracted with DCM and the combined DCM layers were washed with brine solution and dried with Na_2SO_4. After concentration, 40 gm of crude compound was collected.

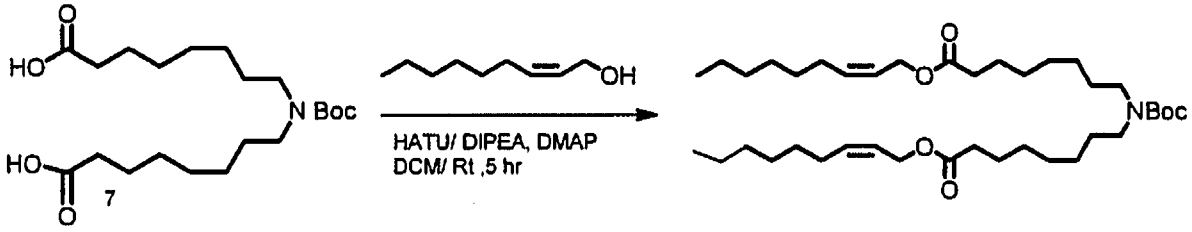
[0151] Crude reaction product was purified by column chromatography using 0-12% ethyl acetate in hexane. The yield recovered was 48%. A single product migrated by thin layer chromatography in 20% ethyl acetate in hexane with an Rf of 0.5, charring with ninhydrine.

Example 6. Synthesis of 8,8'-((tertbutoxycarbonyl)azanediyl) dioctanoic acid

[0152] Dimethyl 8,8'-((tertbutoxycarbonyl)azanedil) dioctanoate was transferred to THF. A 6N sodium hydroxide solution was added at room temperature. The reaction was maintained with stirring overnight at room temperature.

[0153] Reaction mass was evaporated under vacuum at 25°C to remove THF. The reaction product was acidified with 5N HCl. Ethyl acetate was added to the aqueous layer. The separated organic layer was washed with water and the water layer was re-extracted with ethyl acetate. The combined organic layers were washed with brine solution and dried over anhydrous Na_2SO_4. Concentration of the solution gave 18 gm of crude mass.

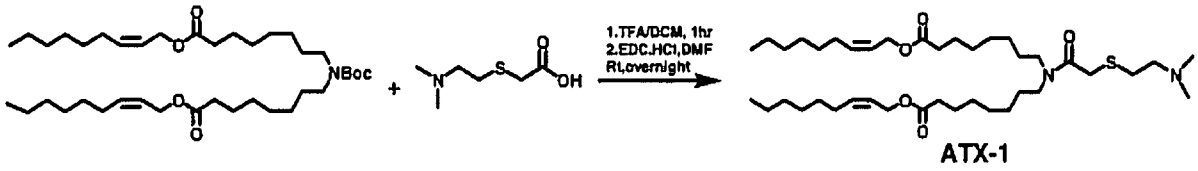
Example 7. Synthesis of di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl)



[0154] 8,8'-((tertbutoxycarbonyl)azanediyl) dioctanoic acid was dissolved in dry DCM. HATU was added to this solution. Di-isopropyl ethyl amine was added slowly to the reaction mixture at room temperature. The internal temp rose to 40°C and a pale yellow color solution was formed. DMAP was added to the reaction mixture followed by cis-2-nonene-1-ol solution in dry DCM. The reaction changed to brown color. The reaction was stirred for five hours at room temperature.

[0155] The reaction was checked by thin layer chromatography under completion. Water was added to the reaction product, which was extracted with DCM. The DCM layer was washed with water followed by brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain 35 gm of crude compound.

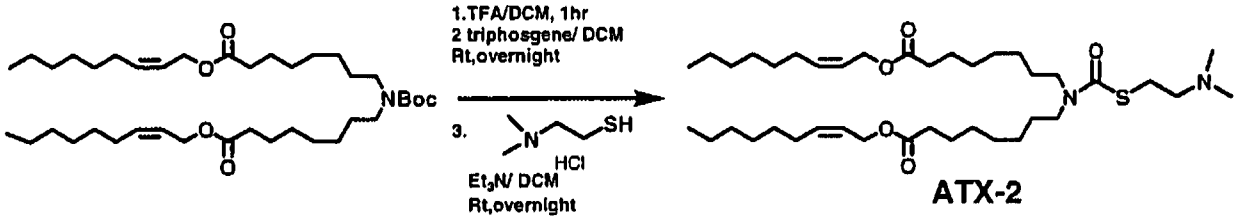
Example 8. Synthesis of ATX-001



ATX-1

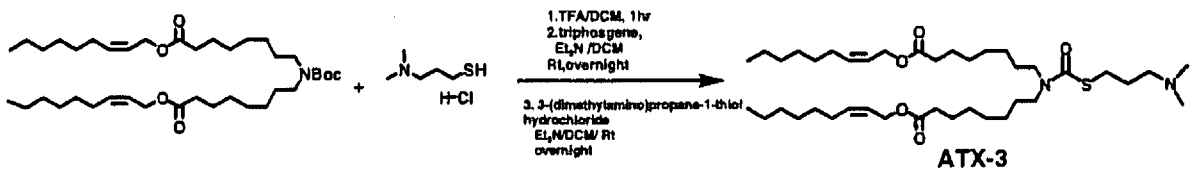
[0156] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (0.023 mol, 15 g) was dissolved in dry dichloromethane (DCM) (200 ml). Trifluoroacetic acid (TFA) was added at 0°C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. The collected crude product (12 grams) was dissolved in dry DCM (85 ml) under nitrogen gas. Triphosgene were added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. DCM solvent was removed from the reaction mass by distillation under N_2. The reaction product was cooled to 0° C, diluted with DCM (50 ml), and 2-((2-(dimethylamino)ethyl)thio) acetic acid (0.039 mol, 6.4g) and carbodiimide (EDC·HCl) (0.054 mol, 10.4 g). The reaction mixture was then stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was diluted with 0.3M HCl solution (75 ml), and the organic layer was separated. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution (75ml) and dried over anhydrous Na_2SO_4. Concentration of the solvent gave a crude mass of 10 gram. The crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 10.5 g (68%).

Example 9. Synthesis of ATX-002



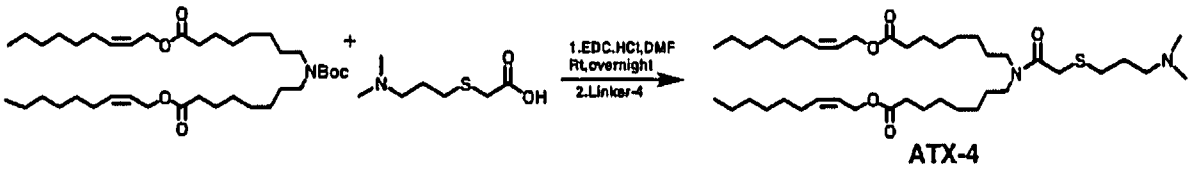
[0157] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (13.85 mmol, 9 grams) was dissolved in dry DCM (150 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. The collected crude product was dissolved in dry DCM (85 ml) under nitrogen gas. Triphosgene were added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. DCM solvent was removed from the reaction mass by distillation under N_2. The reaction product was cooled to 0° C, diluted with DCM (50 ml), and 2-(dimethylamino)ethanethiol HCl (0.063 mol, 8.3 g) was added, followed by Et_3N (dry). The reaction mixture was then stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was diluted with 0.3M HCl solution (75 ml), and the organic layer was separated. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution (75 ml) and dried over anhydrous Na_2SO_4. Concentration of the solvent gave a crude mass of 10 gram. The crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 3.1 gram.

Example 10. Synthesis of ATX-003



[0158] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (0.00337 mol, 2.2 g) was dissolved in dry DCM (20 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated under reduced pressure. The collected crude product was dissolved in dry DCM (10 ml) under nitrogen gas. Triphosgene (0.0182 mol, 5.4 g) was added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. DCM solvent was removed from the reaction mass by distillation under N_2. The reaction product was cooled to 0° C, diluted with DCM (15 ml), and 2-(dimethylamino)propanethiol·HCl (0.0182 mol, 2.82 g) was added, followed by Et_3N (dry). The reaction mixture was then stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was diluted with 0.3 M HCl aqueous solution (20 ml), and the organic layer was separated. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution (50 ml) and dried over anhydrous Na_2SO_4. Concentration of the solvent gave a crude mass of 5 gram. The crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 0.9 gram.

Example 11. Synthesis of ATX-004

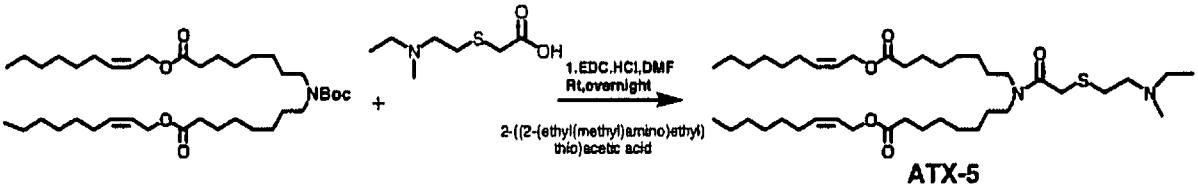


ATX-4

[0159] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (0.023 mol, 15 g) was dissolved in DCM (200 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and

the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. The collected crude product, di((Z)-non-2-en-1-yl)8,8'-azanediyldioctanoate (5.853 mmol, 3.2 g) was dissolved in dry dimethyl formamide (DMF) under nitrogen, and 2-((3-(dimethylamino)propyl)thio)acetic acid (10.48 mmol, 1.85 g) and EDC·HCl (14.56 mmol, 2.78 g) was added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with water (30 ml) and diluted with DCM (30 ml), and the organic layer was separated. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution and dried over anhydrous Na_2SO_4. The crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 1 gram (24.2%).

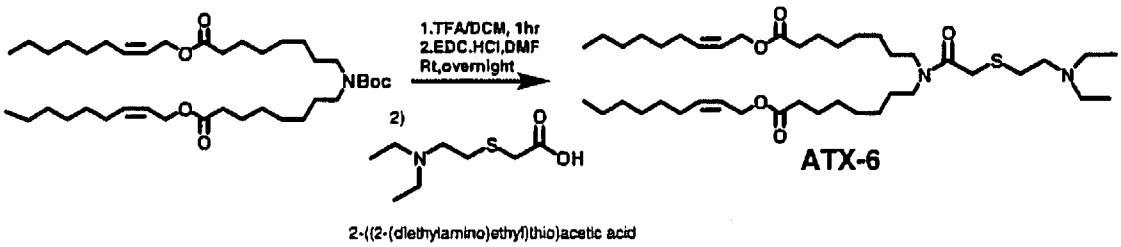
Example 12. Synthesis of ATX-005



[0160] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (0.023 mol, 15 g) was dissolved in dry DCM (200 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. Crude reaction product, di((Z)-non-2-en-1-yl)8,8'-azanediyldioctanoate (5.853 mmol, 3.2 g) was dissolved in dimethylformamide (DMF) under nitrogen gas. 2-((3-(dimethylamino)propyl)thio)acetic acid (10.48 mmol, 1.85 g) and EDC·HCl (14.56 mmol, 2.78 g) were added and the reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was quenched with water (30 ml) and diluted with DCM (30 ml). The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution (75 ml) and dried over anhydrous Na_2SO_4. Concentration of the solvent gave a crude mass of 5 grams.

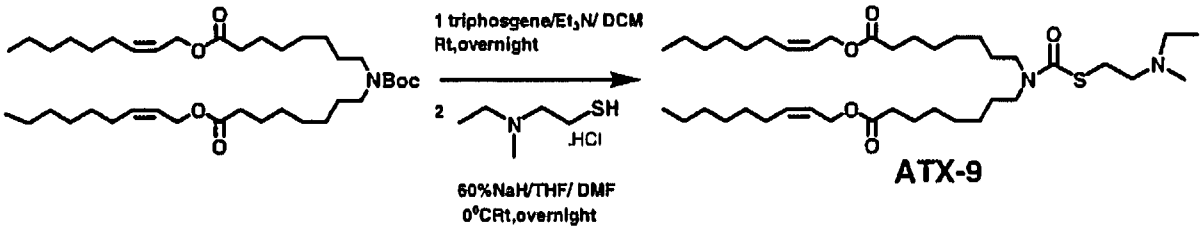
Crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 1 gram (24.2%).

Example 13. Synthesis of ATX-006



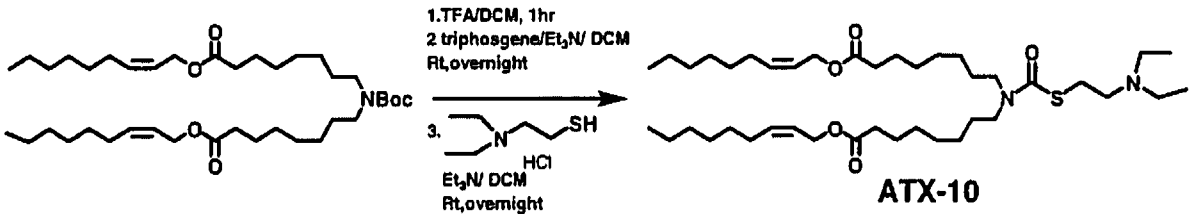
[0161] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate was dissolved in dry DCM (150 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. The collected crude product was dissolved in dry DCM (85 ml) under nitrogen gas. Triphosgene were added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The crude reaction product was dissolved in dry DMF under nitrogen atmosphere, and 2-((2-(diethylamino)ethyl)thio)acetic acid (3.93 mmol, 751 mg) and EDC·HCl (5.45 mmol, 1.0 g) were added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with water (3 ml) and excess DMF was removed under vacuum at 25° C. The reaction product was diluted with water and aqueous layer was extracted thrice with DCM (20 ml). The combined organic layers were washed with brine solution and dried over anhydrous Na_2SO_4 . Concentration of the solvent gave a crude mass of 2 gram. After purification by silica gel column (100-200 mesh) using 3% MeOH/DCM., the yield was 1.2 grams (76%).

Example 14. Synthesis of ATX-009



[0162] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (13.85 mmol, 9 grams) was dissolved in dry DCM (20 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. Di((Z)-non-2-en-1-yl)8,8'-azanediyldioctanoate (0.909 mmol, 500 mg) was dissolved in dry DCM (20 ml) under nitrogen atmosphere. Triphosgene were added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. DCM solvent was removed from the reaction mass by distillation under nitrogen atmosphere. 2-(ethyl(methyl)amino)ethane-1-thiol hydrochloride (4.575 mmol, 715 mg) was dissolved in DMF (7 ml) and tetrahydrofuran (THF) (5 ml), and was added drop wise to the sodium hydride suspension in THF at 0° C. The reaction mixture was then stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was diluted with ethyl acetate and cold water. The reaction was neutralized with 5% HCl (9 ml), and the organic layer was separated. The aqueous layer was re-extracted with ethyl acetate (EtOAc) (20 ml), washed in cold water and brine, and the combined organic layers were washed and dried over anhydrous Na_2SO_4. Concentration of the solvent gave 1 gram or crude product. The compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM to yield 100 mg.

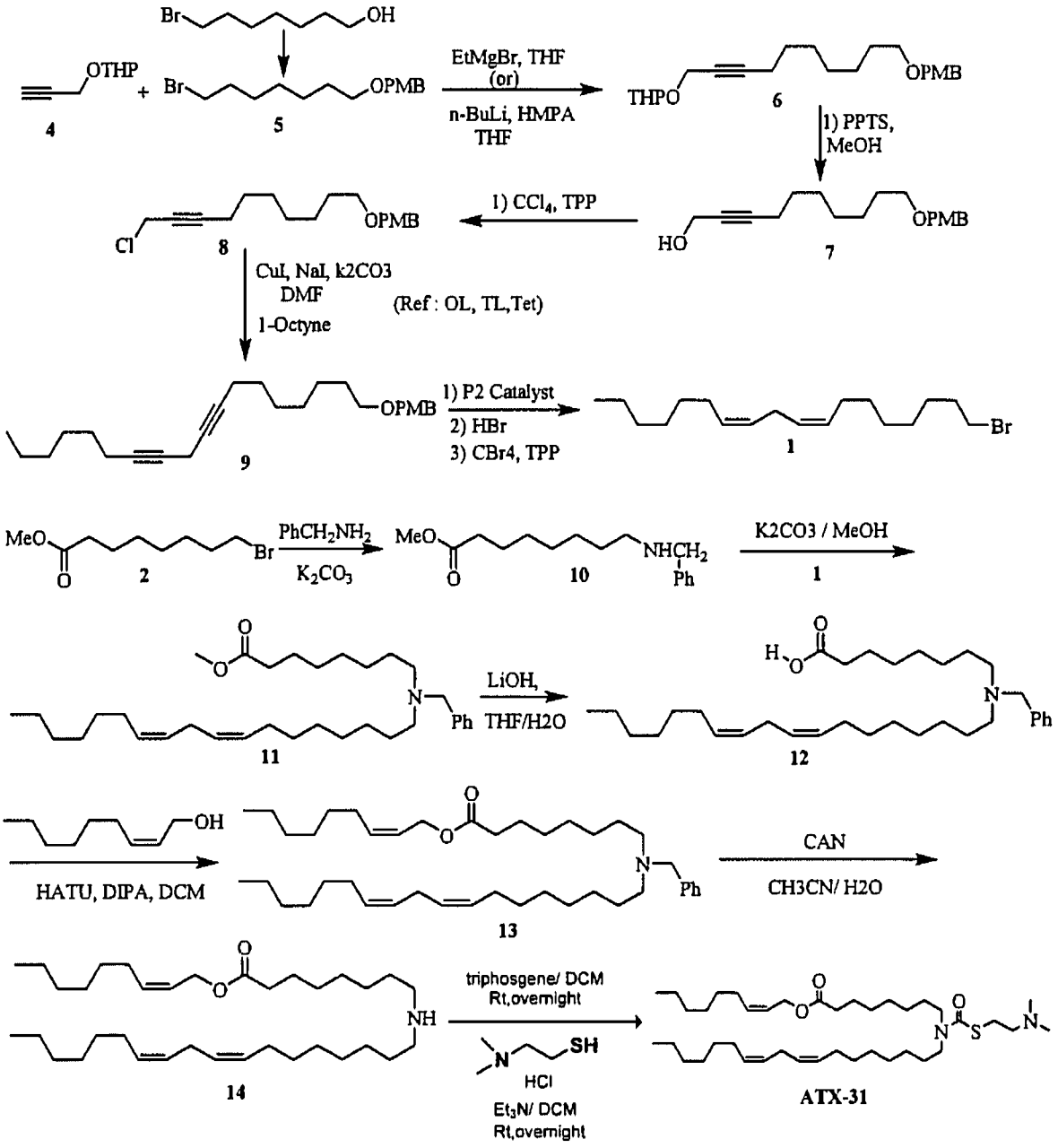
Example 15. Synthesis of ATX-010



[0163] Di((Z)-non-2-en-1-yl) 8,8'((tertbutoxycarbonyl)azanediyl) dioctanoate (3.079 mmol, 2 g) was dissolved in dry DCM (20 ml). TFA was added at 0° C to initiate a reaction. The reaction temperature was slowly allowed to warm to room temperature for 30 minutes with stirring. Thin layer chromatography showed that the reaction was completed. The reaction product was concentrated under vacuum at 40° C and the crude residue was diluted with DCM, and washed with a 10% $NaHCO_3$ solution. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated. The collected crude product was dissolved in dry DCM (20 ml) under nitrogen gas. Triphosgene (14.55 mmol, 4.32 g) was added and the reaction mixture was cooled to 0° C, and Et_3N was added drop wise. The reaction mixture was stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. DCM solvent was removed from the reaction mass by distillation under N_2. The reaction product was cooled to 0° C, diluted with DCM (20 ml), and 2-(dimethylamino)ethanethiol HCl (0.063 mol, 8.3 g) was added, followed by Et_3N (dry). The reaction mixture was then stirred overnight at room temperature. Thin layer chromatography showed that the reaction was completed. The reaction product was diluted with 0.3 M HCl solution (20 ml), and the organic layer was separated. The aqueous layer was re-extracted with DCM, and the combined organic layers were washed with 10% K_2CO_3 aqueous solution 20 ml) and dried over anhydrous Na_2SO_4. Concentration of the solvent gave a crude mass of 10 gram. The crude compound was purified by silica gel column (100-200 mesh) using 3% MeOH/DCM. The yield was 1.4 g (75%).

Example 16. Synthesis of ATX-A-4 (referred to below as ATX-031)

[0164]



Example 17. Synthesis of ATX-011 to ATX-017, ATX-021 to ATX-023, and ATX-026 to ATX-030 from Table 1, and the compounds of Tables 2, 3, and 4

[0165] The synthesis of ATX-011 to ATX-017, ATX-021 to ATX-023, and ATX-026 to ATX-030, ATX-A-1 to ATX-A-22 and the compounds of Tables 2, 3, and 4 follows the synthesis of Examples 1-15, by substituting appropriate starting ingredients for synthetic reactions described therein.

Example 18. *In vivo* mouse Factor VII silencing

[0166] Using a liver-directed *in vivo* screen of the liposome libraries, a series of compounds were tested that facilitate high levels of siRNA mediated gene silencing in hepatocytes, the cells comprising the liver parenchyma. Factor VII, a blood clotting factor, is a suitable target gene for assaying functional siRNA delivery to liver. Because this factor is produced specifically in hepatocytes, gene silencing indicates successful delivery to parenchyma, as opposed to delivery to the cells of the reticulo-endothelial system (e.g., Kupffer cells). Furthermore, Factor VII is a secreted protein that can be readily measured in serum, obviating the need to euthanize animals. Silencing at the mRNA level can be readily determined by measuring levels of protein. This is because the protein's short half-life (2–5 hour). C57BL/6 mice (Charles River Labs) received either saline or siRNA in liposome formulations via tail vein injection at a volume of 0.006 ml/g. At 48 h after administration, animals were anesthetized by isofluorane inhalation and blood was collected into serum separator tubes by retroorbital bleed. Serum levels of Factor VII protein were determined in samples using a chromogenic assay (Biophen FVII, Aniara Corporation) according to manufacturers' protocols. A standard curve was generated using serum collected from saline-treated animals.

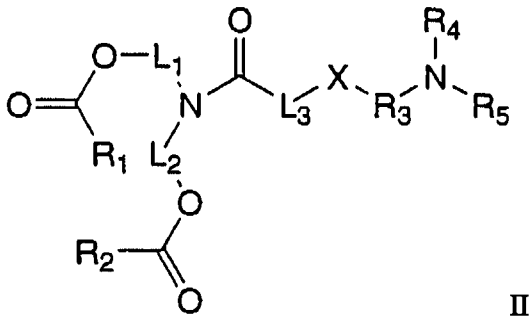
[0167] Compositions with siRNA directed to Factor VIII were formulated with ATX-001, ATX-002, ATX-003, and ATX-547, and comparator samples NC1 and MC3 (Alnylam). These were injected into animals at 0.3 mg/kg and at 1 mg/kg. The siRNA encapsulated by MC3 (0.3 mg/kg), NC1 (0.3 mg/kg), ATX-547 (0.3 mg/kg), ATX-001 (0.3 and 1.0 mg/kg), ATX-002 (0.3 and 1.0 mg/kg), and ATX-003 (0.3 and 1.0 mg/kg) was measured for the ability to knockdown Factor VII in mouse plasma following administration of the siRNA formulation to C57BL6 mice. The results showed that ATX-001 and ATX-002 were most effective at 0.3 mg/kg, compared to controls (Figs. 1 and 2).

[0168] The siRNA encapsulated MC3 (0.3 and 1.5 mg/kg), NC1 (0.3 mg/kg), ATX-547 (0.1 and 0.3 mg/kg), ATX-004 (0.3), ATX-006 (0.3 and 1.0 mg/kg), ATX-010 (0.3 mg/kg), and ATX-001 (0.3 and 1.5 mg/kg), was measured for Factor VII knockdown in mouse plasma

following administration of the siRNA formulation to C57BL6 mice. The results showed that ATX-001 and ATX-010 were most effective (Figs. 3 and 4). The knockdown activity of the exemplary compounds is shown for 0.3 mg/kg or at 0.05 mg/kg for ATX-018, ATX-019, and ATX-020 (Table 1).

What is Claimed:

1. A compound of formula II



II

wherein

R_1 and R_2 both consist of a linear alkyl consisting of 1 to 12 carbons, or an alkenyl or alkynyl consisting of 2 to 12 carbons;

L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N;

X is S;

L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or forming a heterocycle with N;

R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and

R_4 and R_5 are the same or different, each consisting of a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein L_1 and L_2 both consist of a linear alkylene consisting of five carbons.

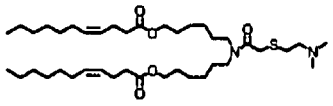
3. The compound of claim 1, wherein R_3 consists of ethylene or propylene.

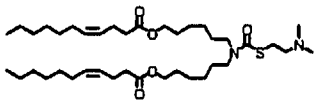
4. The compound of claim 1, wherein R_4 and R_5 are the same or different, each consisting of hydrogen, methyl, or ethyl.

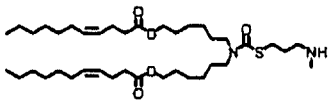
5. The compound of claim 1, wherein L_3 consists of a bond.

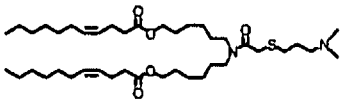
6. The compound of claim 1, wherein R_1 and R_2 both consist of a linear alkenyl consisting of ten carbons.

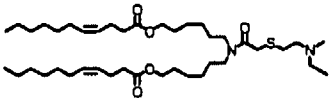
7. The compound of claim 1, selected from a compound of formulas 1) to 12)

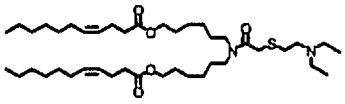
1) 

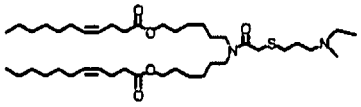
2) 

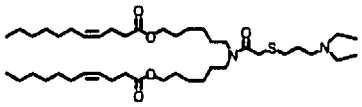
3) 

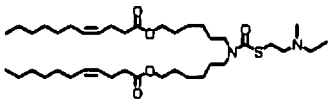
4) 

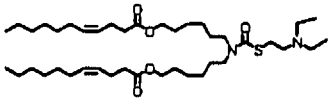
5) 

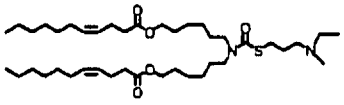
6) 

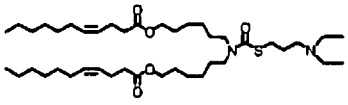
7) 

8) 

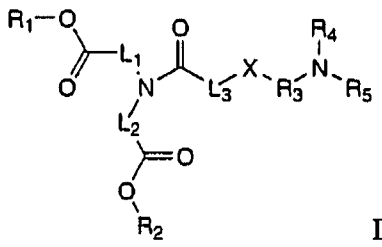
9) 

10) 

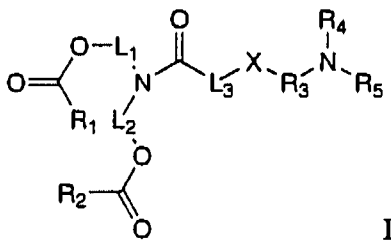
11) 

12) 

8. A compound of formula III or IV



III



IV

wherein

R_1 consists of a branched alkyl with 12 to 20 carbons,

R_2 consists of a linear alkyl with 5 to 10 carbons or a branched alkyl with 12 to 20 carbons,

L_1 and L_2 each consist of a bond or a linear alkyl having 1 to 3 carbon atoms,

X consists of S or O,

L_3 consists of a bond or a lower alkyl,

R_3 consists of a lower alkyl, and

R_4 and R_5 are the same or different, each consisting of a lower alkyl;

or a pharmaceutically acceptable salt thereof.

9. The compound of claim 8, wherein L_3 consists of a bond.

10. The compound of claim 8, wherein X consists of S.

11. The compound of claim 8, wherein R_3 consists of ethylene.

12. The compound of claim 8, wherein R_3 consists of n-propylene or isopropylene.

13. The compound of claim 8, wherein R_4 and R_5 each consist of methyl, ethyl, or isopropyl.

14. The compound of claim 8, wherein L_1 and L_2 both consist of a bond.

15. The compound of claim 8, wherein L_1 and L_2 both consist of a methylene.

16. The compound of claim 8, wherein R_2 consists of an alkyl.

17. The compound of claim 8, wherein R_1 and R_2 both consist of branched alkyl.

18. The compound of claim 17, wherein the branched alkyl consists of 19 or 20 carbon atoms.

19. The compound of claim 17, wherein the branched alkyl consists of 13 or 14 carbon atoms.

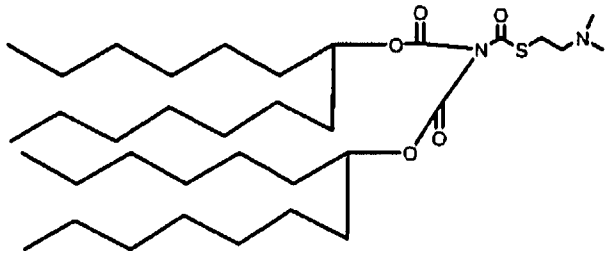
20. The compound of claim 8, wherein L_3 consists of methylene, R_3 consists of ethylene, X_2 consists of S, and R_4 and R_5 both consist of methyl.

21. The compound of claim 8, wherein L_3 consists of a bond, R_3 consists of ethylene, X consists of S, and R_4 and R_5 both consist of methyl.

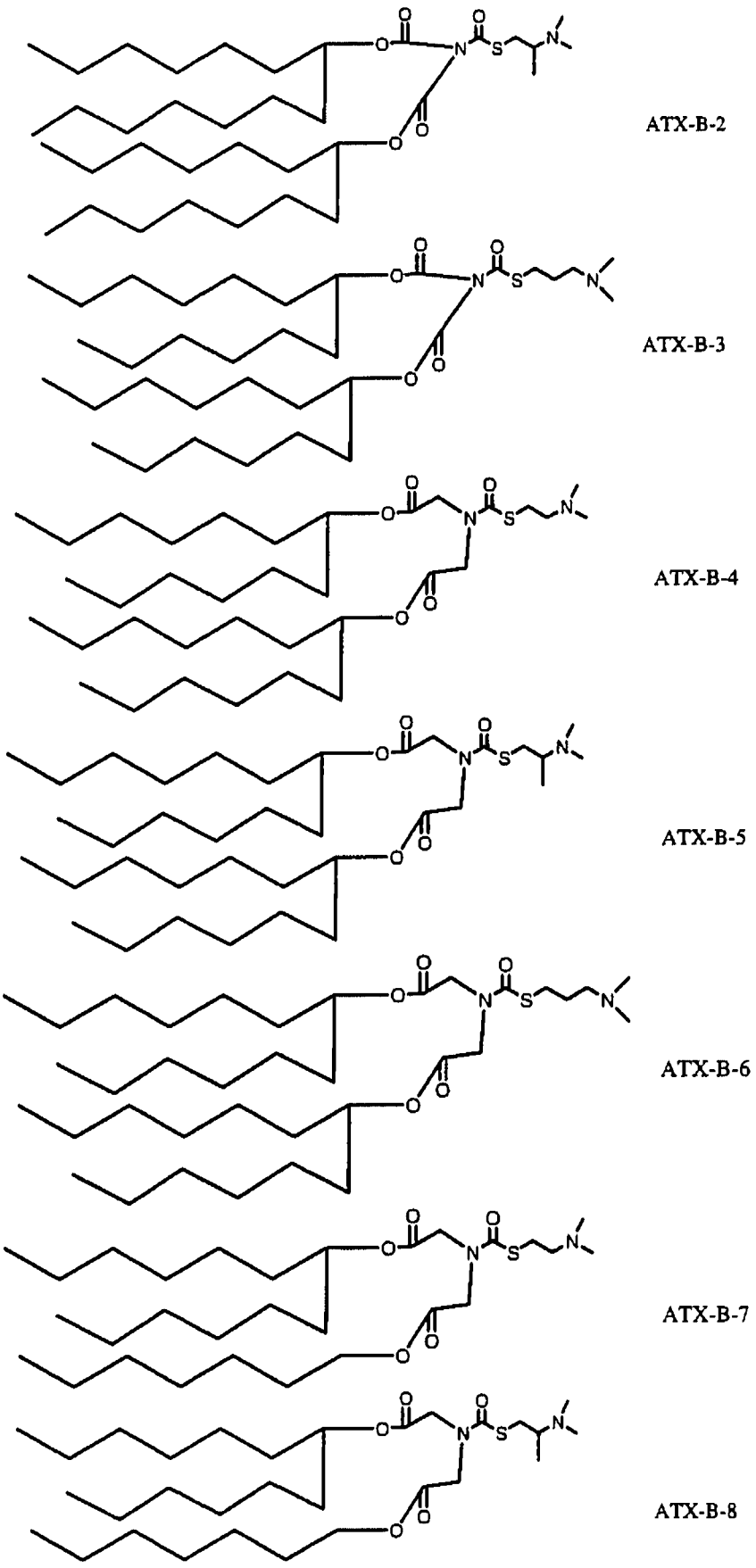
22. The compound of claim 8, wherein L_3 consists of a bond, R_3 consists of n-propylene, X consists of S, and R_4 and R_5 both consist of methyl.

23. The compound of claim 8, wherein L_3 consists of a bond, R_3 consists of isopropylene, X consists of S, and R_4 and R_5 both consist of methyl.

24. The compound of claim 8, selected from a compound of formula ATX-B-1 to ATX-B-12



ATX-B-1



ATX-B-2

ATX-B-3

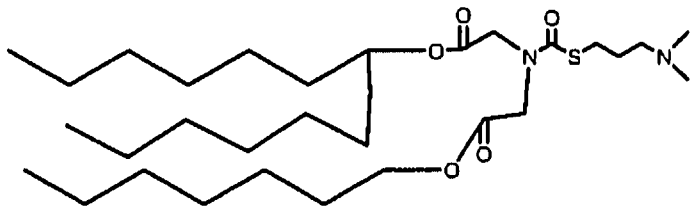
ATX-B-4

ATX-B-5

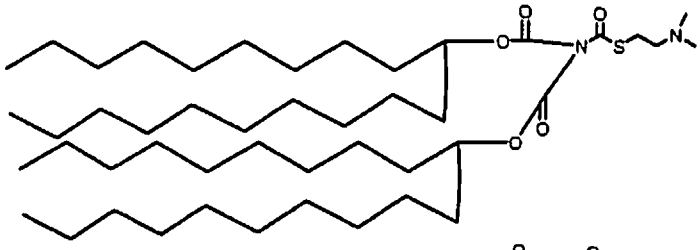
ATX-B-6

ATX-B-7

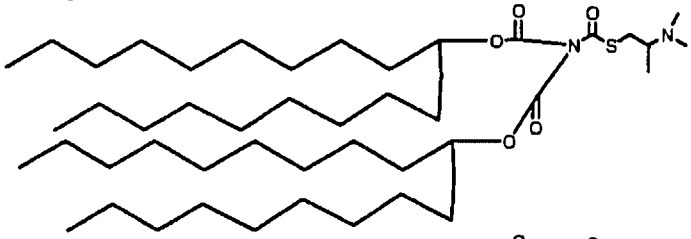
ATX-B-8



ATX-B-9



ATX-B-10



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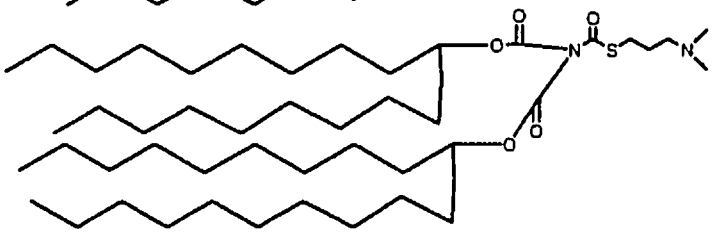

ATX-B-12

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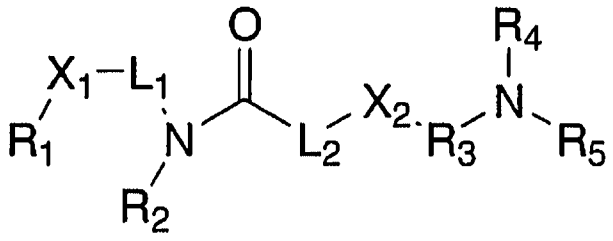


V

wherein

R_1 consists of a linear or branched alkyl consisting of 1-18 carbons, or an alkenyl or alkynyl consisting of 2 to 12 carbons, or a cholesteryl;

R_2 consists of a linear or branched alkyl or an alkenyl consisting of 1 to 18 carbons;

L_1 consists of a linear alkyl consisting of 5 to 9 carbons or, when R_1 consists of a cholesteryl, L_1 consists of a linear alkylene or alkenyl consisting of 3 to 4 carbons;

X_1 consists of –O-(CO)— or –(CO)-O—;

X_2 consists of S or O;

L_2 consists of a bond or a linear alkylene of 1 to 6 carbons;

R_3 consists of a linear or branched alkylene with 1 to 6 carbons; and

R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

26. The compound of claim 25, wherein X_1 consists of –O-(CO)—.

27. The compound of claim 25, wherein X_2 consists of S.

28. The compound of claim 25, wherein R_3 consists of ethylene.

29. The compound of claim 25, wherein R_3 consists of n-propylene or isopropylene.

30. The compound of claim 25, wherein R_4 and R_5 each consist of methyl, ethyl, or isopropyl.

31. The compound of claim 25, wherein L_2 consists of a bond.

32. The compound of claim 25, wherein L_2 consists of a methylene.

33. The compound of claim 25, wherein R_2 consists of an alkyl.

34. The compound of claim 25, wherein R_1 and R_2 both consist of branched alkyl.

35. The compound of claim 34, wherein the branched alkyl consists of 19 or 20 carbon atoms.

36. The compound of claim 34, wherein the branched alkyl consists of 13 or 14 carbon atoms.

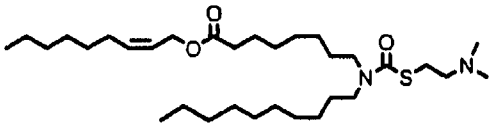
37. The compound of claim 25, wherein L_2 consists of methylene, R_3 consists of ethylene, X_1 consists of –O-(CO)—, X_2 is S, and R_4 and R_5 both consist of methyl.

38. The compound of claim 25, wherein L_2 consists of a bond, R_3 consists of ethylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

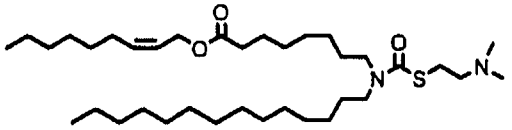
39. The compound of claim 25, wherein L_2 consists of a bond, R_3 consists of n-propylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

40. The compound of claim 25, wherein L_2 consists of a bond, R_3 consists of isopropylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

41. The compound of claim 25, selected from the group consisting of the compounds of formula ATX-A-1 to ATX-A-22



ATX-A-1



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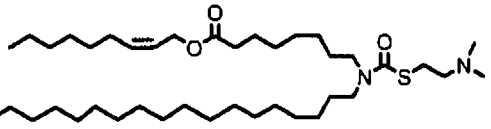

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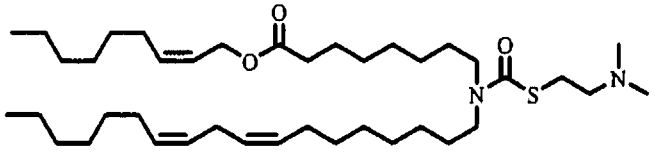

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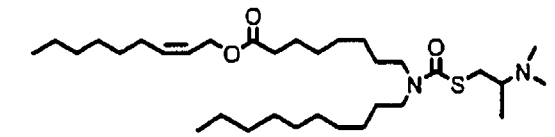

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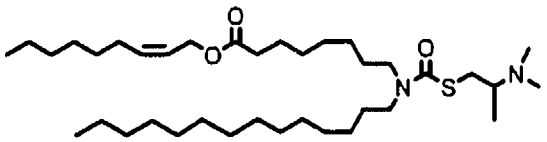

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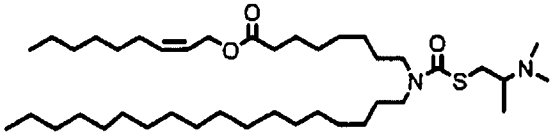

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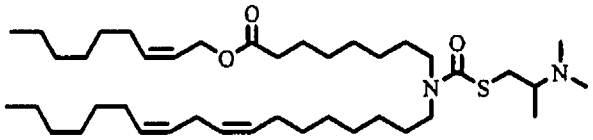

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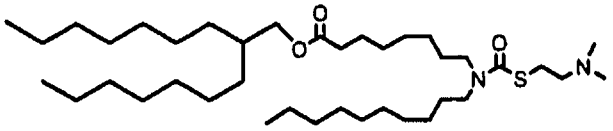

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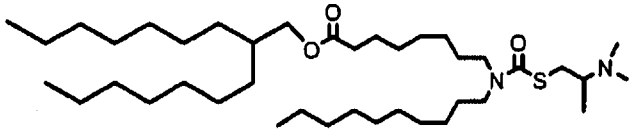

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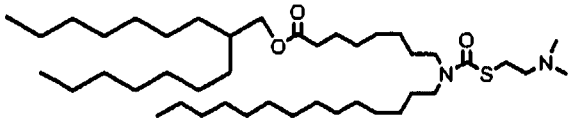

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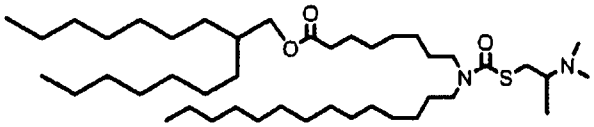

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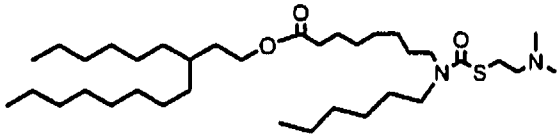

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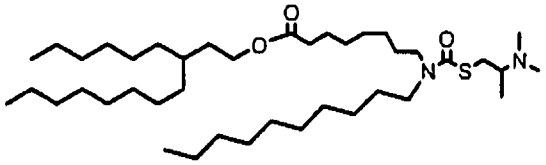

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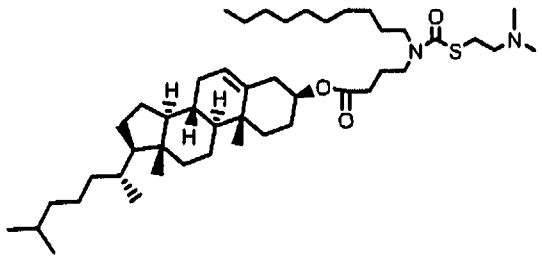

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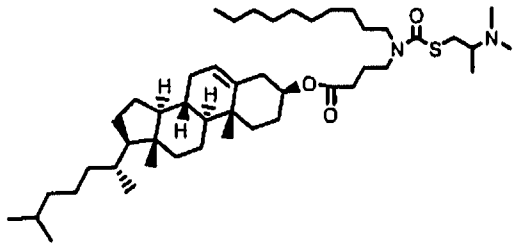

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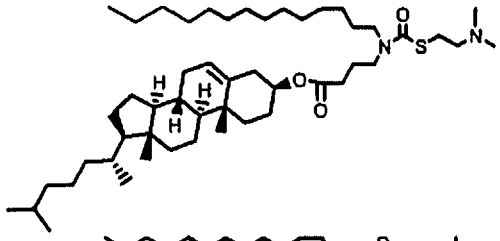

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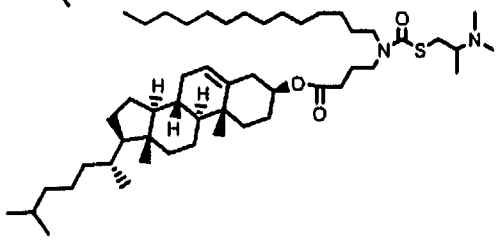

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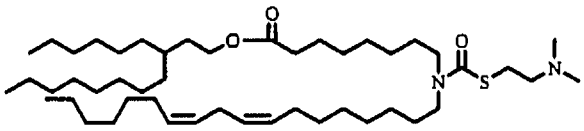

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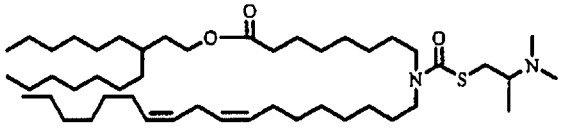

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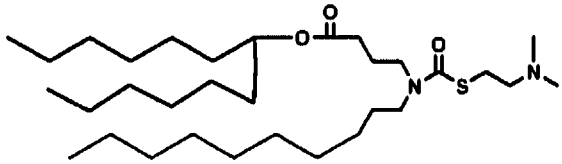

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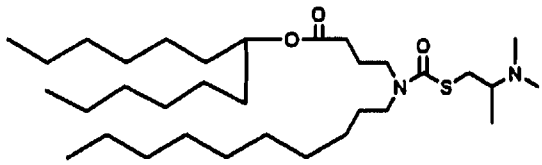
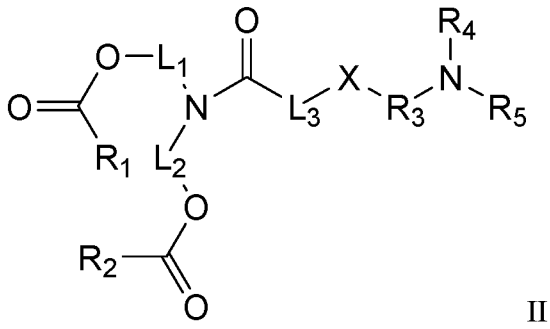


ATX-A-22

AMENDED CLAIMS
received by the International Bureau on 18 March 2016 (18.03.2016)

1. A compound of formula II



II

wherein

R_1 and R_2 both consist of a linear alkyl consisting of 1 to 14 carbons, or an alkenyl or alkynyl consisting of 2 to 14 carbons;

L_1 and L_2 both consist of a linear alkylene or alkenylene consisting of 5 to 18 carbons, or forming a heterocycle with N;

X is S;

L_3 consists of a bond or a linear alkylene consisting of 1 to 6 carbons, or forming a heterocycle with N;

R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons; and

R_4 and R_5 are the same or different, each consisting of a hydrogen or a linear or branched alkyl consisting of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein R_1 and R_2 both are an alkenyl.

3. The compound of claim 1, wherein R_1 and R_2 both are an alkyl.

4. The compound of claim 1, wherein L_1 and L_2 both are a linear alkylene.

5. The compound of claim 1, wherein L_1 and L_2 both are an alkenylene.

6. The compound of claim 1, wherein L_1 and L_2 both consist of a linear alkylene consisting of five carbons.

7. The compound of claim 1, wherein R_3 consists of ethylene or propylene.

8. The compound of claim 1, wherein R_4 and R_5 each consist of hydrogen, methyl, or ethyl.

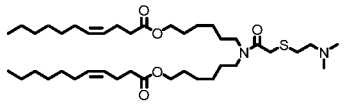
9. The compound of claim 1, wherein L_3 consists of a bond.

10. The compound of claim 1, wherein L_3 consists of a linear alkylene.

11. The compound of claim 1, wherein R_1 and R_2 both consist of a linear alkenyl consisting of ten carbons.

12. The compound of claim 1, selected from a compound of formulas 1) to 12)

1)



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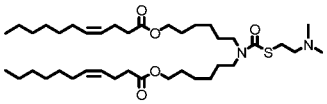

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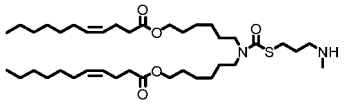

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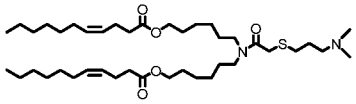

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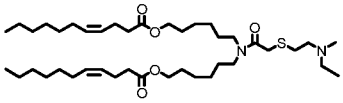

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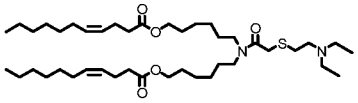
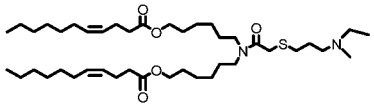


7)



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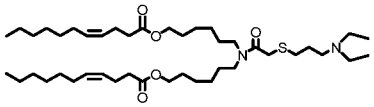

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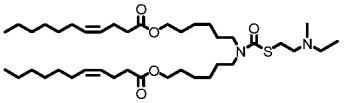

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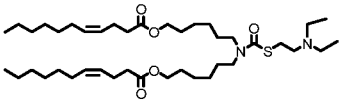

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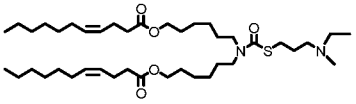

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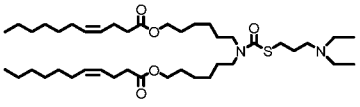
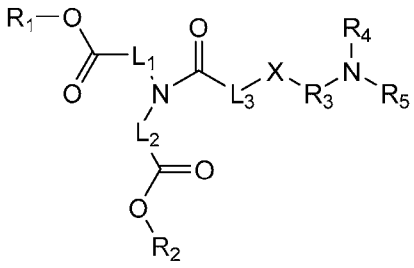
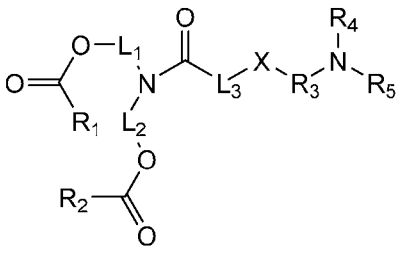


13. A compound of formula III or IV



III



IV

wherein

R_1 consists of a branched alkyl with 12 to 20 carbons,

R_2 consists of a linear alkyl with 5 to 10 carbons or a branched alkyl with 12 to 20 carbons,

L_1 and L_2 each consist of a bond or a linear alkyl having 1 to 3 carbon atoms,

X consists of S or O,

L_3 consists of a bond or an alkylene consisting of 1 to 6 carbons,

R_3 consists of a linear or branched alkylene consisting of 1 to 6 carbons, and

R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl consisting of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

14. The compound of claim 13, wherein L_3 consists of a bond.

15. The compound of claim 17, wherein L_3 consists of an alkylene.

16. The compound of claim 13, wherein X consists of S.

17. The compound of claim 13, wherein R_3 consists of ethylene.

18. The compound of claim 13, wherein R_3 consists of n-propylene or isopropylene.

19. The compound of claim 13, wherein R_4 and R_5 each consist of methyl, ethyl, or isopropyl.

20. The compound of claim 13, wherein L_1 and L_2 each consist of a bond.

21. The compound of claim 13, wherein L_1 and L_2 each consist of a linear alkylene.

22. The compound of claim 13, wherein L_1 and L_2 each consist of a methylene.

23. The compound of claim 13, wherein R_2 consists of a linear alkyl.

24. The compound of claim 13, wherein R_2 consists of a branched alkyl.

25. The compound of claim 24, wherein the branched alkyl consists of 19 or 20 carbon atoms.

26. The compound of claim 24, wherein the branched alkyl consists of 13 or 14 carbon atoms.

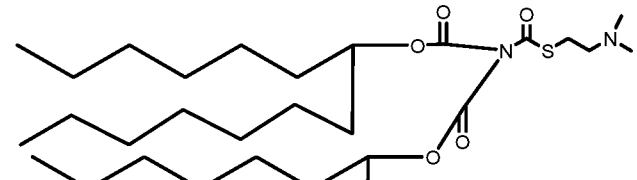
27. The compound of claim 13, wherein L_3 consists of methylene, R_3 consists of ethylene, X_2 consists of S, and R_4 and R_5 both consist of methyl.

28. The compound of claim 13, wherein L_3 consists of a bond, R_3 consists of ethylene, X consists of S, and R_4 and R_5 both consist of methyl.

29. The compound of claim 13, wherein L_3 consists of a bond, R_3 consists of n-propylene, X consists of S, and R_4 and R_5 both consist of methyl.

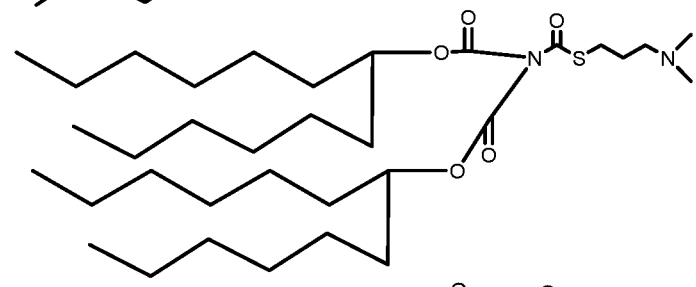
30. The compound of claim 13, wherein L_3 consists of a bond, R_3 consists of isopropylene, X consists of S, and R_4 and R_5 both consist of methyl.

31. The compound of claim 13, selected from a compound of formula ATX-B-1 to ATX-B-12

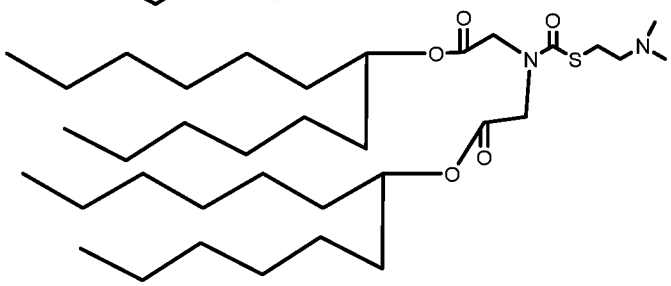


ATX-B-1

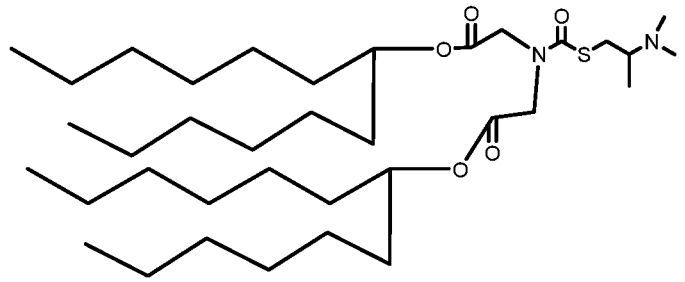
ATX-B-2



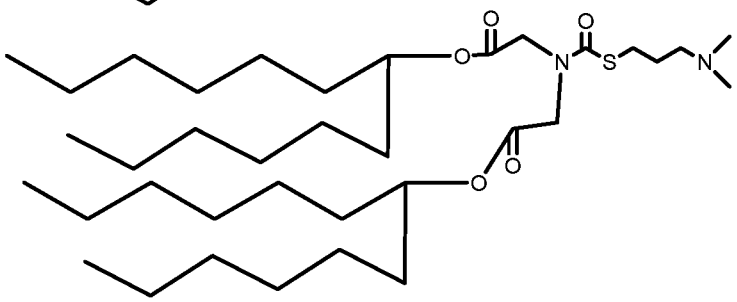
ATX-B-3



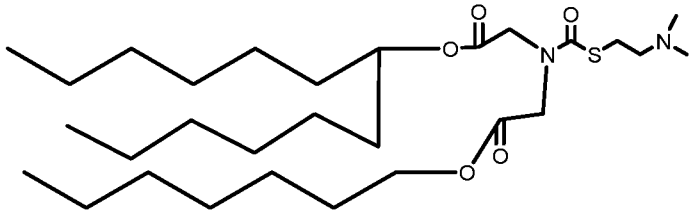
ATX-B-4



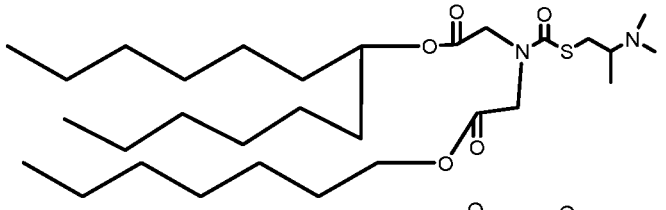
ATX-B-5



ATX-B-6



ATX-B-7



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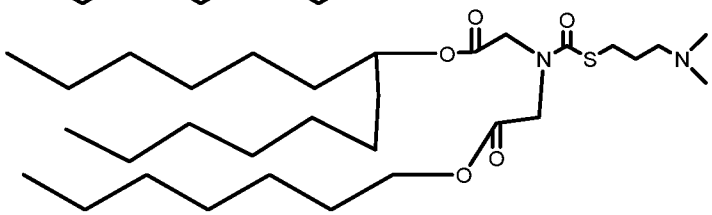
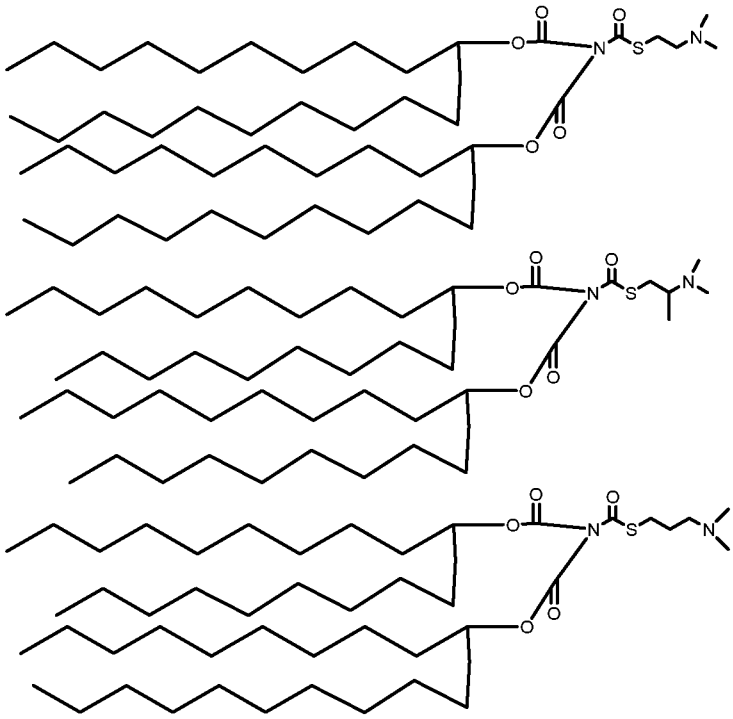


ATX-B-9

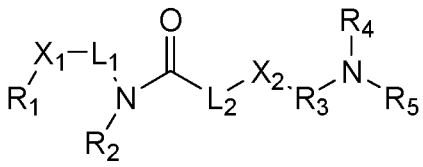


ATX-B-10

ATX-B-11

ATX-B-12

32. A compound of formula V



V

wherein

R_1 consists of a linear or branched alkyl consisting of 1 to 20 carbons, or an alkenyl or alkynyl consisting of 2 to 12 carbons, or a cholesteryl;

R_2 consists of a linear or branched alkyl consisting of 1 to 20 carbons or an alkenyl consisting of 2 to 20 carbons;

L_1 consists of a linear alkyl consisting of 3 to 9 carbons or, when R_1 consists of a cholesteryl, L_1 consists of a linear alkylene or alkenyl consisting of 3 to 4 carbons;

X_1 consists of –O-(CO)— or –(CO)-O—;

X_2 consists of S or O;

L_2 consists of a bond or a linear alkylene of 1 to 6 carbons;

R_3 consists of a linear or branched alkylene with 1 to 6 carbons; and

R_4 and R_5 are the same or different, each consisting of a linear or branched alkyl of 1 to 6 carbons;

or a pharmaceutically acceptable salt thereof.

33. The compound of claim 32, wherein X_1 consists of –O-(CO)—.

34. The compound of claim 32, wherein X_2 consists of S.

35. The compound of claim 32, wherein R_3 consists of ethylene.

36. The compound of claim 32, wherein R_3 consists of n-propylene or isopropylene.

37. The compound of claim 32, wherein R_4 and R_5 each consist of methyl, ethyl, or isopropyl.

38. The compound of claim 32, wherein L_2 consists of a bond.

39. The compound of claim 32, wherein L_2 consists of a methylene.

40. The compound of claim 32, wherein R_2 consists of an alkyl.

41. The compound of claim 32, wherein R_1 and R_2 both consist of a branched alkyl.

42. The compound of claim 41, wherein the branched alkyl consists of 19 or 20 carbon atoms.

43. The compound of claim 41, wherein the branched alkyl consists of 13 or 14 carbon atoms.

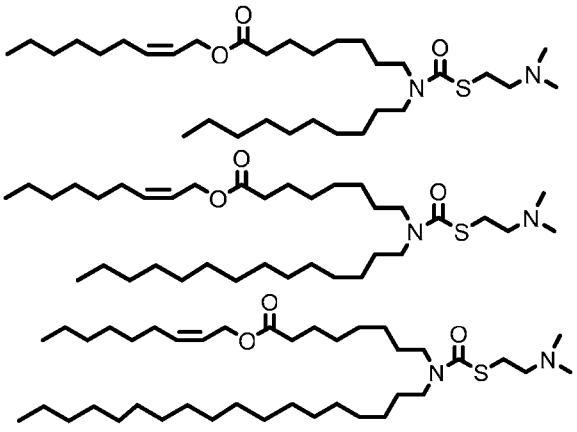
44. The compound of claim 32, wherein L_2 consists of methylene, R_3 consists of ethylene, X_1 consists of –O-(CO)—, X_2 is S, and R_4 and R_5 both consist of methyl.

45. The compound of claim 32, wherein L_2 consists of a bond, R_3 consists of ethylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

46. The compound of claim 32, wherein L_2 consists of a bond, R_3 consists of n-propylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

47. The compound of claim 32wherein L_2 consists of a bond, R_3 consists of isopropylene, X_1 consists of –O-(CO)—, X_2 consists of S, and R_4 and R_5 both consist of methyl.

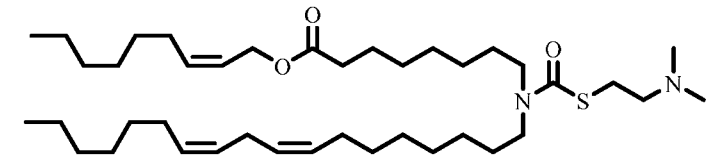
48. The compound of claim 32, selected from the group consisting of the compounds of formula ATX-A-1 to ATX-A-22



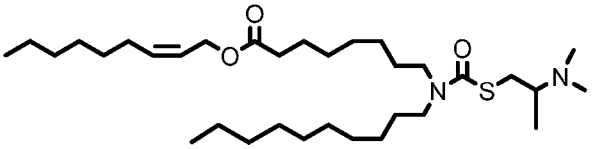
ATX-A-1

ATX-A-2

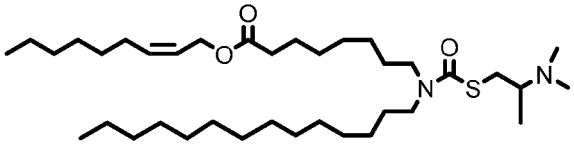
ATX-A-3



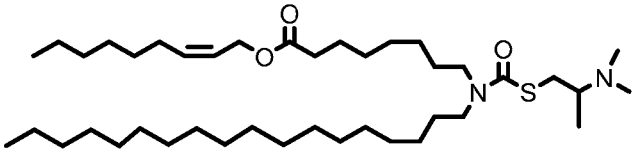
ATX-A-4



ATX-A-5



ATX-A-6



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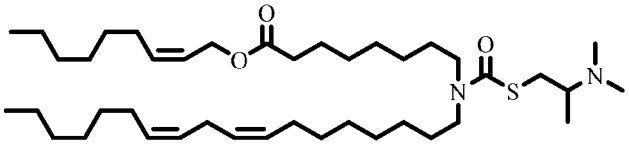

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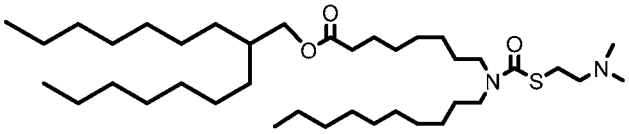

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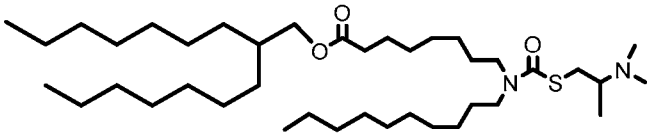

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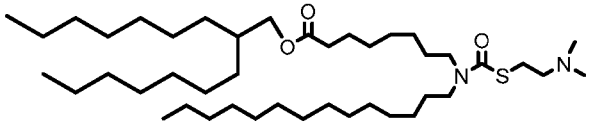

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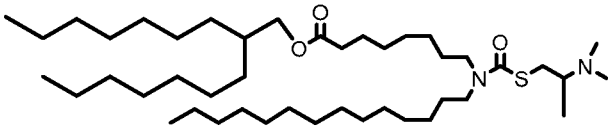

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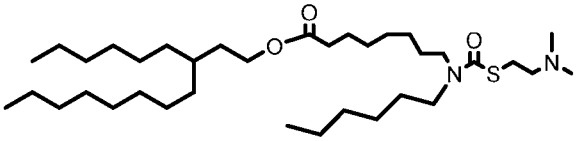
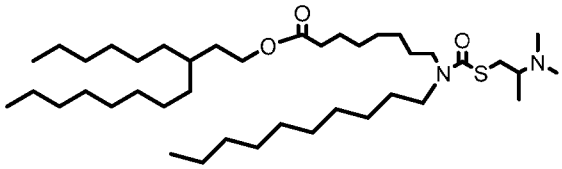
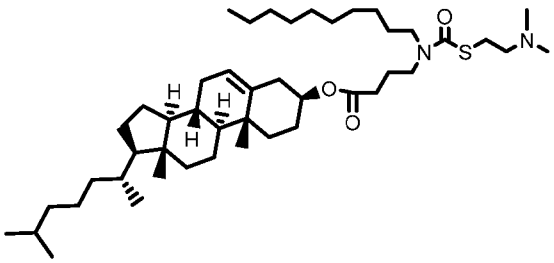


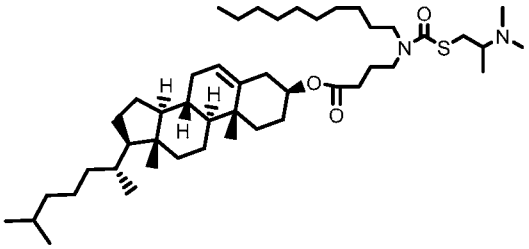
ATX-A-13



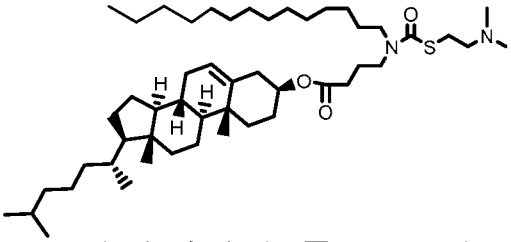
ATX-A-14



ATX-A-15



ATX-A-16



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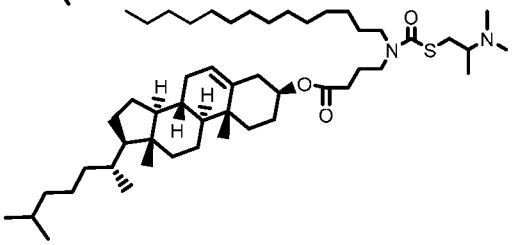
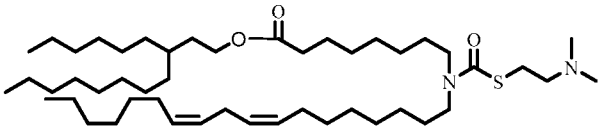
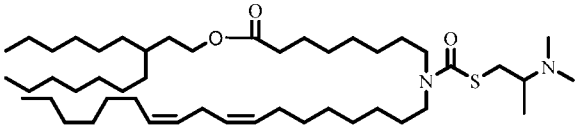


ATX-A-18



ATX-A-19



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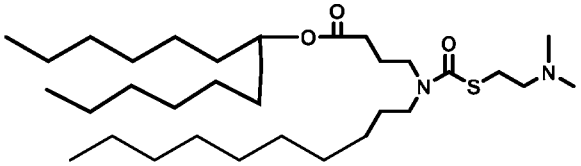

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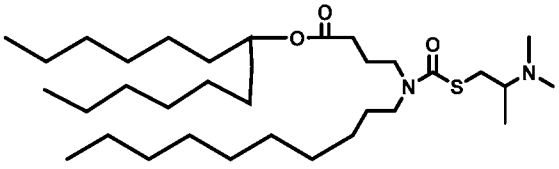

ATX-A-22

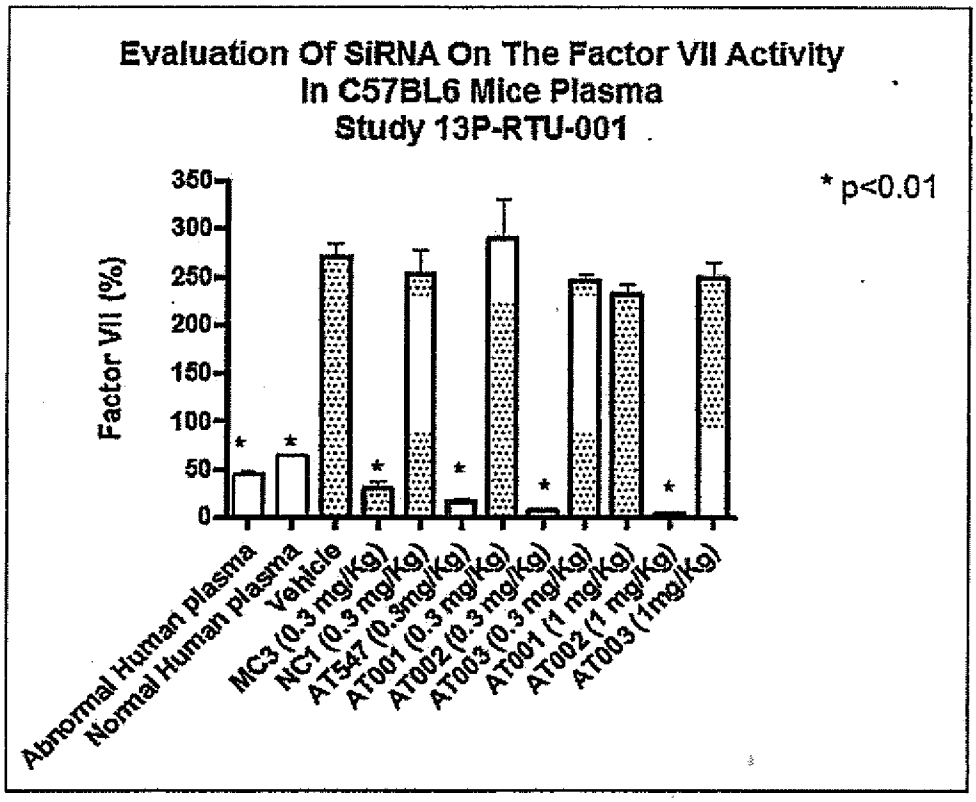
Evaluation Of SiRNA On The Factor VII Activity
In C57BL6 Mice Plasma
Study 13P-RTU-001
* p<0.01
Factor VII (%)
350
300
250
200
150
100
50
0
Abnormal Human plasma
Normal Human plasma
Vehicle
MC3 (0.3 mg/Kg)
NC1 (0.3 mg/Kg)
AT547 (0.3mg/Kg)
AT001 (0.3 mg/Kg)
AT002 (0.3 mg/Kg)
AT003 (0.3 mg/Kg)
AT001 (1 mg/Kg)
AT002 (1 mg/Kg)
AT003 (1mg/Kg)

Fig. 1

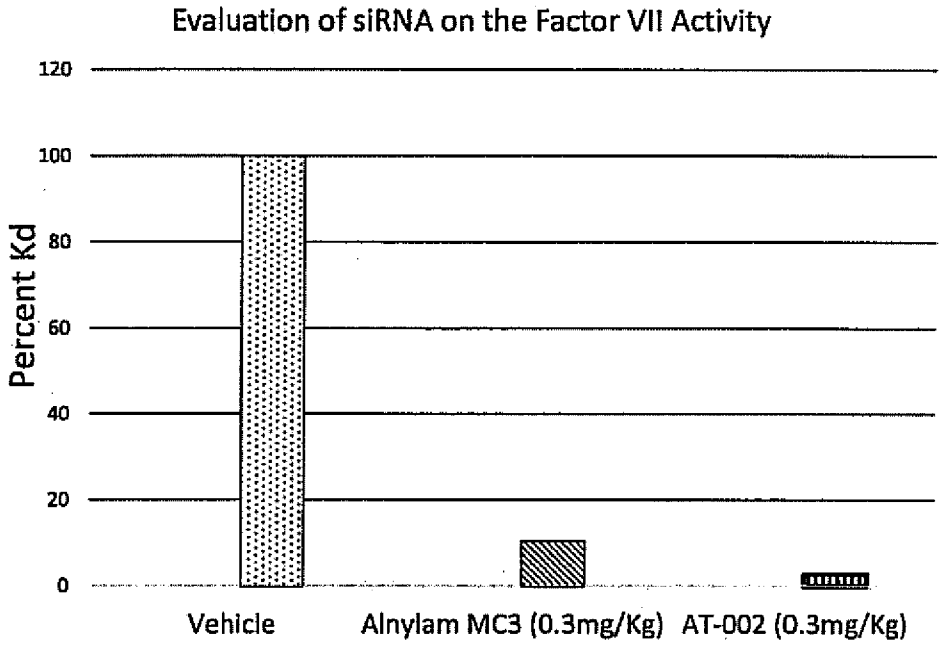
Evaluation of siRNA on the Factor VII Activity
Percent Kd
120
100
80
60
40
20
0
Vehicle
Alnylam MC3 (0.3mg/Kg)
AT-002 (0.3mg/Kg)

Fig. 2

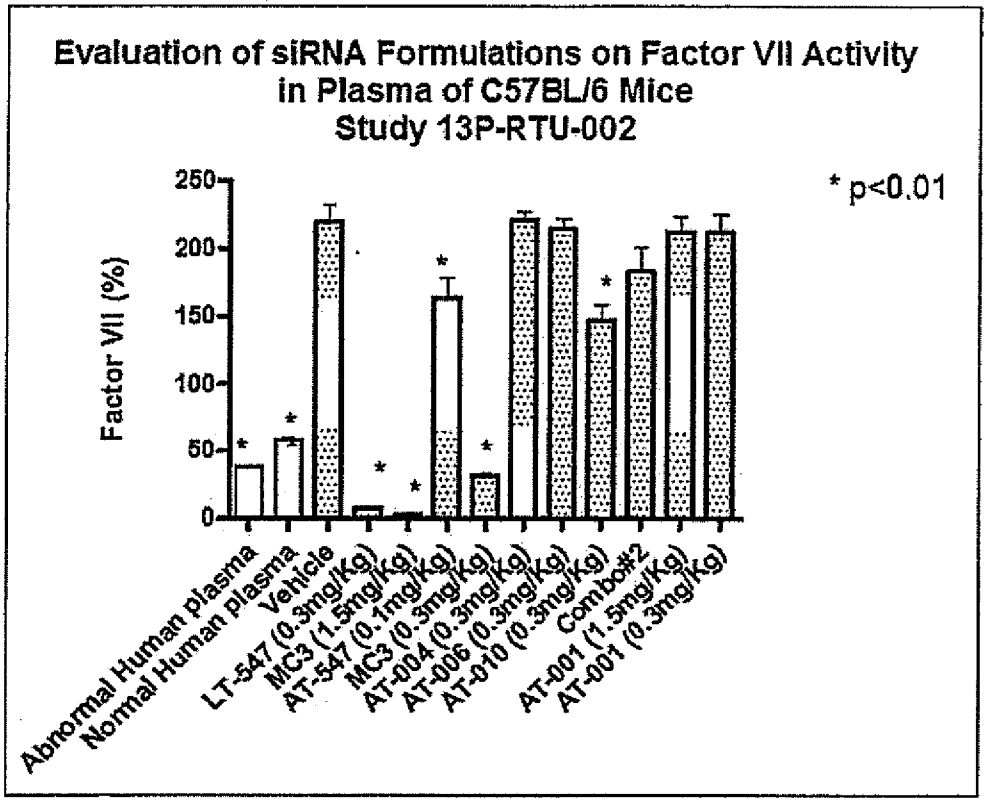
Evaluation of siRNA Formulations on Factor VII Activity
in Plasma of C57BL/6 Mice
Study 13P-RTU-002
* p<0.01
Factor VII (%)
250
200
150
100
50
0
Abnormal Human plasma
Normal Human plasma
Vehicle
LT-547 (0.3mg/Kg)
MC3 (1.5mg/Kg)
AT-547 (0.1mg/Kg)
MC3 (0.3mg/Kg)
AT-004 (0.3mg/Kg)
AT-006 (0.3mg/Kg)
AT-010 (0.3mg/Kg)
Combo#2
AT-001 (1.5mg/Kg)
AT-001 (0.3mg/Kg)

Fig. 3

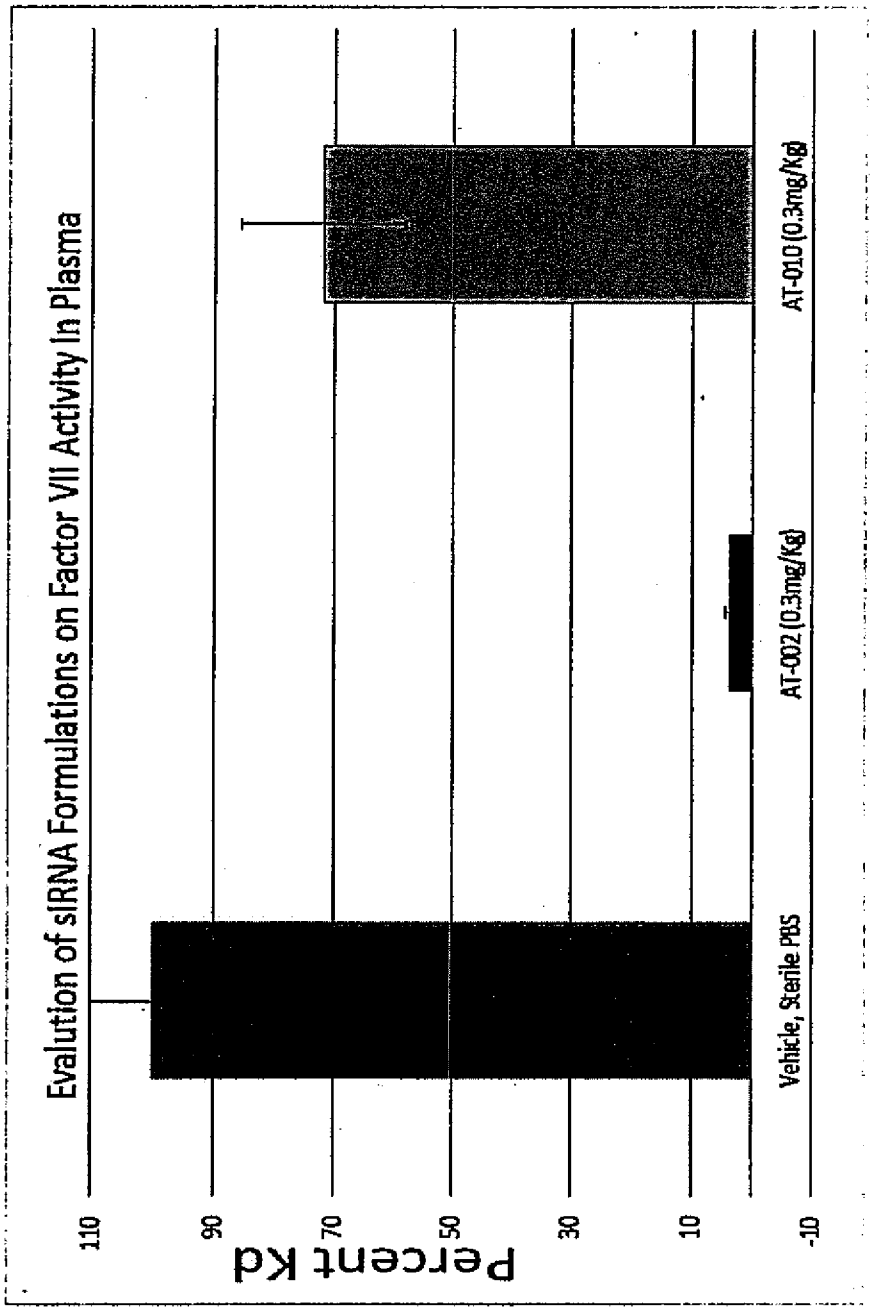
Evalution of siRNA Formulations on Factor VII Activity In Plasma
Percent Kd
110
90
70
50
30
10
-10
Vehicle, Sterile PBS
AT-002 (0.3mg/Kg)
AT-010 (0.3mg/Kg)

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/030218

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C323/60 C07C333/04 C07C333/10 C07J31/00
ADD. A61K9/127 C12N15/11

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C A61K C07J C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/086373 A1 (ALNYLAM PHARMACEUTICALS INC [US]) 13 June 2013 (2013-06-13) claims 59,61-72,74-78,80-83	1-24
Y	WO 2013/185116 A1 (PAYNE JOSEPH E [US]; GAUDETTE JOHN A [US]; HOU ZHENG [US]; AHMADIAN MO) 12 December 2013 (2013-12-12) paragraphs [0117] - [0118], [0123] - [0127], [0137] - [0143], [0145] - [0152], [0155] - [0163]; table 1	1-24
E	WO 2015/074085 A1 (ARCTURUS THERAPEUTICS INC [US]) 21 May 2015 (2015-05-21) claim 24; compounds ATX-018, ATX-019, ATX-020, ATX031	25-28, 30,31, 33,38,41

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

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2015	25/08/2015

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

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/030218

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013086373 A1	13-06-2013	US 2014308304 A1	16-10-2014
		WO 2013086373 A1	13-06-2013
WO 2013185116 A1	12-12-2013	AU 2013270685 A1	29-01-2015
		CA 2876148 A1	12-12-2013
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		EP 2858974 A1	15-04-2015
		KR 20150021566 A	02-03-2015
		TW 201402146 A	16-01-2014
		US 2013330401 A1	12-12-2013
		WO 2013185116 A1	12-12-2013
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		WO 2015074085 A1	21-05-2015

(19)中华人民共和国国家知识产权局

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(43)申请公布日 2017.09.26

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(71)申请人 阿克丘勒斯治疗公司
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(74)专利代理机构 北京集佳知识产权代理有限公司 11227
代理人 郑斌 刘振佳
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C07C 333/04(2006.01)
C07C 333/10(2006.01)
C07J 1/00(2006.01)
A61K 9/127(2006.01)
C12N 15/11(2006.01)

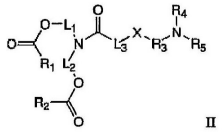
权利要求书8页 说明书41页 附图3页
按照条约第19条修改的权利要求书8页

(54)发明名称

用于RNA递送的可电离阳离子脂质

(57)摘要

所描述的是式II、III、IV和V化合物。式II化合物由以下化合物组成，其中R_1和R_2都是由1至12个碳组成的直链烷基、由2至12个碳组成的烯基或炔基；L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；X是S；L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；R_3由1至6个碳所组成的直链或支链亚烷基组成；并且R_4和R_5是相同或不同的，各自由氢或1至6个碳所组成的直链或支链烷基组成。式III和IV化合物由以下化合物组成，其中R_1由具有12至20个碳的支链烷基组成；R_2由具有5至10个碳的直链烷基或具有12至20个碳的支链烷基组成；L_1和L_2各自由键或具有1至3个碳原子的直链烷基组成；X由S或O组成；L_3由键或低碳烷基组成；R_3由低碳烷基组成；并且R_4和R_5是相同或不同的，各自由低碳烷基组成。式V化合物由以下化合物组成，其中R_1由1-18个碳所组成的直链或支链烷基、2至12个碳所组成的烯基或炔基、或胆固醇基组成；R_2由1至18个碳所组成的606338680v1直链或支链烷基或烯基组成；L_1由5至9个碳所组成的直链烷基组成，或当R_1由胆固醇基组成时，那么L_1由3至4个碳所组成的直链亚烷基或烯基组成；X_1由-O-(CO)-或-(CO)-O-组成；X_2由S或O组成；La由键或具有1至6个碳的直链亚烷基组成；R_3由具有1至6个碳的直链或支链亚烷基组成；并且R_4和R_5是相同或不同的，各自由具有1至6个碳的直链或支链烷基组成。式II、III、IV和V化合物可包含其药学上可接受的盐。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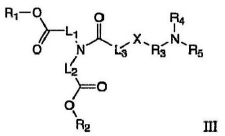
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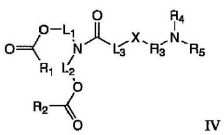
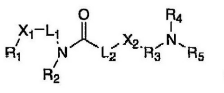
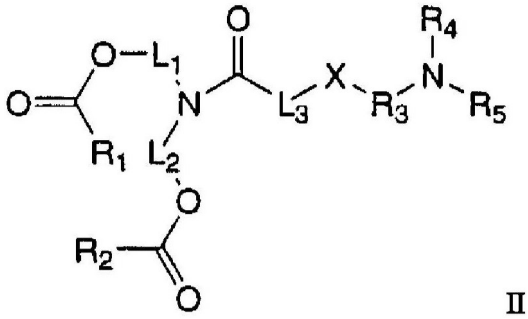

IV


V

CN 107207428 A

1.一种式II化合物，



II

其中

R_1和R_2都由1至12个碳所组成的直链烷基、或2至12个碳所组成的烯基或炔基组成；

L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；

X是S；

L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；

R_3由1至6个碳所组成的直链或支链亚烷基组成；以及

R_4和R_5是相同或不同的，各自由氢或1至6个碳所组成的直链或支链烷基组成；

或其药学上可接受的盐。

2.根据权利要求1所述的化合物，其中L_1和L_2都由五个碳所组成的直链亚烷基组成。

3.根据权利要求1所述的化合物，其中R_3由亚乙基或亚丙基组成。

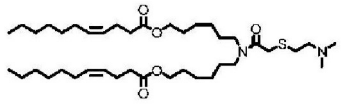
4.根据权利要求1所述的化合物，其中R_4和R_5是相同或不同的，各自由氢、甲基或乙基组成。

5.根据权利要求1所述的化合物，其中L_3由键组成。

6.根据权利要求1所述的化合物，其中R_1和R_2都由十个碳所组成的直链烯基组成。

7.根据权利要求1所述的化合物，其选自式1)至12)的化合物：

1)



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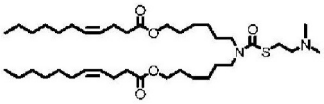

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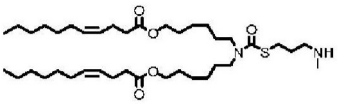

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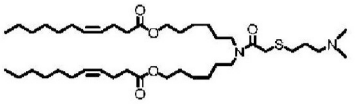

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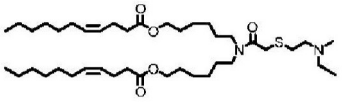

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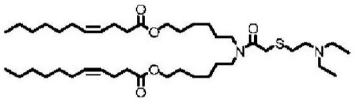

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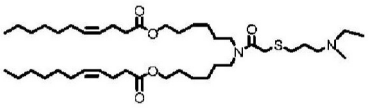

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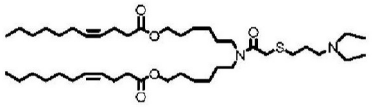

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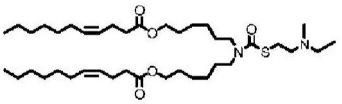
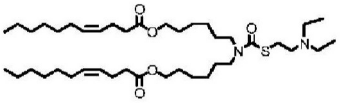
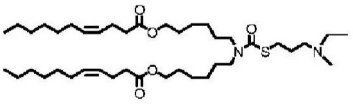


10)



11)



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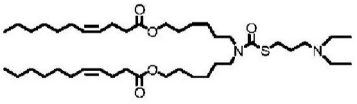
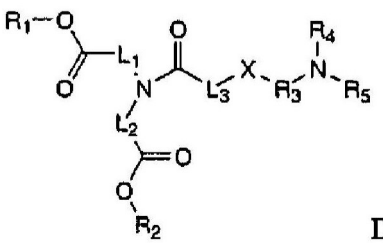
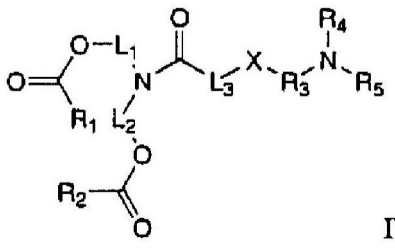


。

8.一种式III或IV化合物，



III



IV

其中

R_1由具有12至20个碳的支链烷基组成，

R_2由具有5至10个碳的直链烷基或具有12至20个碳的支链烷基组成，

L_1和L_2各自由键或具有1至3个碳原子的直链烷基组成，

X由S或O组成，

L_3由键或低碳烷基组成，

R_3由低碳烷基组成，以及

R_4和R_5是相同或不同的，各自由低碳烷基组成；

或其药学上可接受的盐。

9.根据权利要求8所述的化合物，其中L_3由键组成。

10.根据权利要求8所述的化合物，其中X由S组成。

11.根据权利要求8所述的化合物，其中R_3由亚乙基组成。

12.根据权利要求8所述的化合物，其中R_3由亚正丙基或亚异丙基组成。

13.根据权利要求8所述的化合物，其中R_4和R_5各自由甲基、乙基或异丙基组成。

14.根据权利要求8所述的化合物，其中L_1和L_2都由键组成。

15.根据权利要求8所述的化合物，其中L_1和L_2都由亚甲基组成。

16.根据权利要求8所述的化合物，其中R_2由烷基组成。

17.根据权利要求8所述的化合物，其中R_1和R_2都由支链烷基组成。

18.根据权利要求17所述的化合物，其中所述支链烷基由19或20个碳原子组成。

19.根据权利要求17所述的化合物，其中所述支链烷基由13或14个碳原子组成。

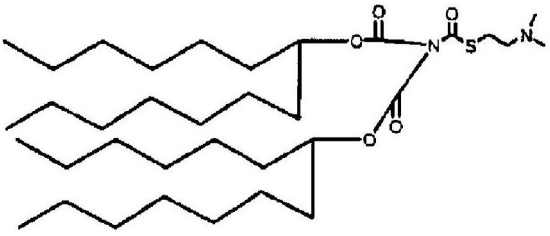
20.根据权利要求8所述的化合物，其中L_3由亚甲基组成，R_3由亚乙基组成，X_2由S组成，并且R_4和R_5都由甲基组成。

21.根据权利要求8所述的化合物，其中L_3由键组成，R_3由亚乙基组成，X由S组成，并且R_4和R_5都由甲基组成。

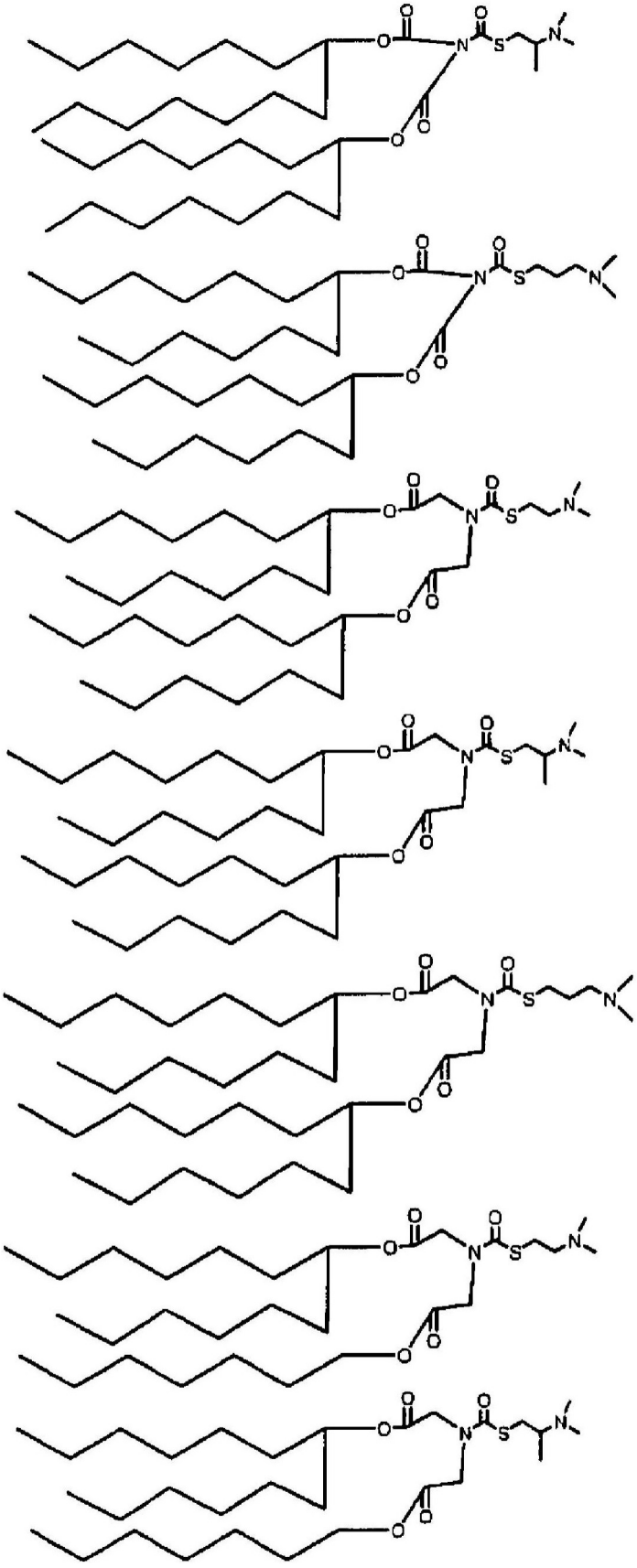
22.根据权利要求8所述的化合物，其中L_3由键组成，R_3由亚正丙基组成，X由S组成，并且R_4和R_5都由甲基组成。

23.根据权利要求8所述的化合物，其中L_3由键组成，R_3由亚异丙基组成，X由S组成，并且R_4和R_5都由甲基组成。

24.根据权利要求8所述的化合物，其选自式ATX-B-1至ATX-B-12的化合物：



ATX-B-1



ATX-B-2

ATX-B-3

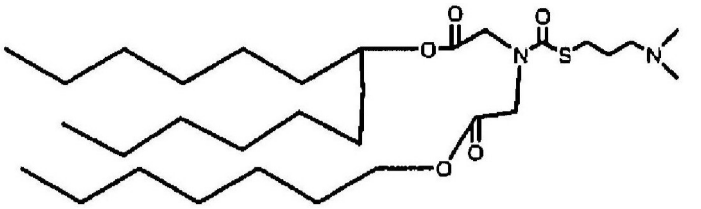
ATX-B-4

ATX-B-5

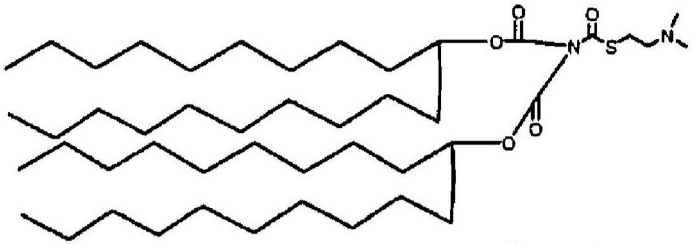
ATX-B-6

ATX-B-7

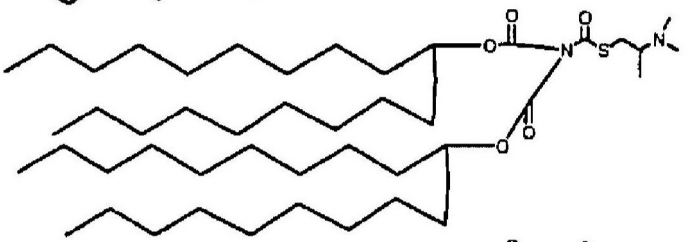
ATX-B-8



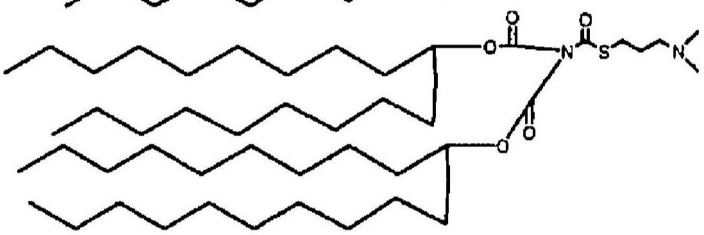
ATX-B-9



ATX-B-10

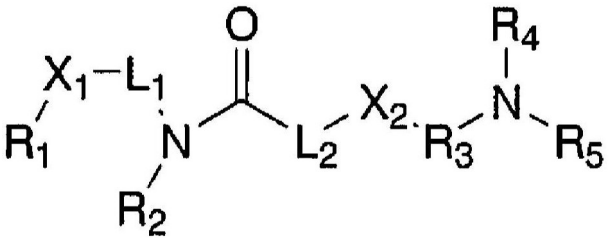


ATX-B-11



ATX-B-12

25.一种式V化合物，



V

其中

R_1由1-18个碳所组成的直链或支链烷基、或2至12个碳所组成的烯基或炔基、或胆固醇基组成；

R_2由1至18个碳所组成的直链或支链烷基或烯基组成；

L_1由5至9个碳所组成的直链烷基组成，或当R_1由胆固醇基组成时，L_1由3至4个碳所组成的直链亚烷基或烯基组成；

X_1由-O-(CO)-或-(CO)-O-组成；

X_2由S或O组成；

L_2由键或具有1至6个碳的直链亚烷基组成；

R_3由具有1至6个碳的直链或支链亚烷基组成；以及

R_4和R_5是相同或不同的，各自由具有1至6个碳的直链或支链烷基组成；

或其药学上可接受的盐。

26.根据权利要求25所述的化合物，其中X_1由-O-(CO)-组成。

27.根据权利要求25所述的化合物，其中X_2由S组成。

28.根据权利要求25所述的化合物，其中R_3由亚乙基组成。

29.根据权利要求25所述的化合物，其中R_3由亚正丙基或亚异丙基组成。

30.根据权利要求25所述的化合物，其中R_4和R_5各自由甲基、乙基或异丙基组成。

31.根据权利要求25所述的化合物，其中L_2由键组成。

32.根据权利要求25所述的化合物，其中L_2由亚甲基组成。

33.根据权利要求25所述的化合物，其中R_2由烷基组成。

34.根据权利要求25所述的化合物，其中R_1和R_2都由支链烷基组成。

35.根据权利要求34所述的化合物，其中所述支链烷基由19或20个碳原子组成。

36.根据权利要求34所述的化合物，其中所述支链烷基由13或14个碳原子组成。

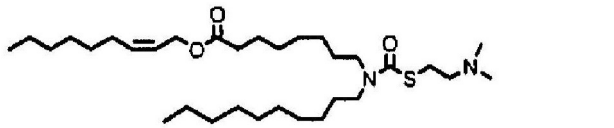
37.根据权利要求25所述的化合物，其中L_2由亚甲基组成，R_3由亚乙基组成，X_1由-O-(CO)-组成，X_2是S，并且R_4和R_5都由甲基组成。

38.根据权利要求25所述的化合物，其中L_2由键组成，R_3由亚乙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

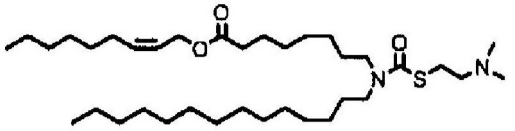
39.根据权利要求25所述的化合物，其中L_2由键组成，R_3由亚正丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

40.根据权利要求25所述的化合物，其中L_2由键组成，R_3由亚异丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

41.根据权利要求25所述的化合物，其选自由式ATX-A-1至ATX-A-22的化合物组成的群组：



ATX-A-1



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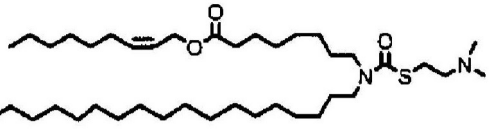

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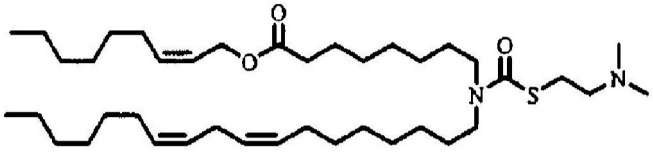
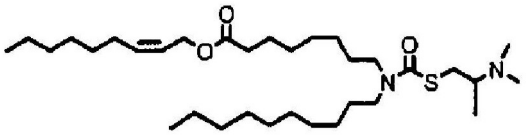
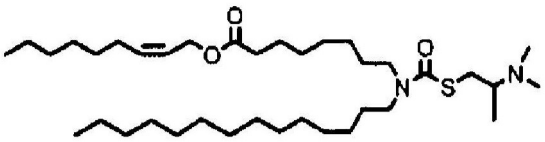


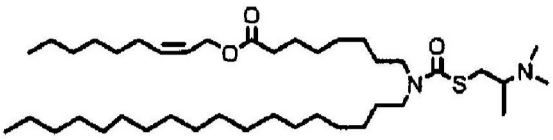
ATX-A-4



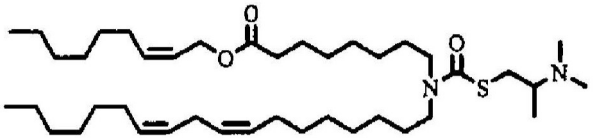
ATX-A-5



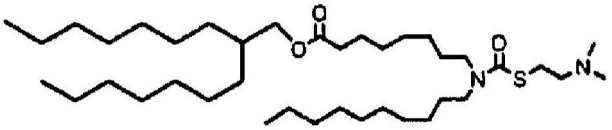
ATX-A-6



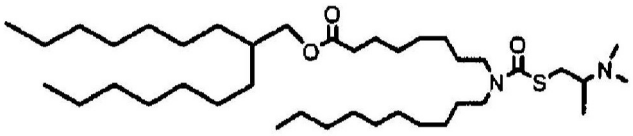
ATX-A-7



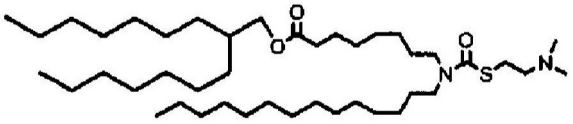
ATX-A-8



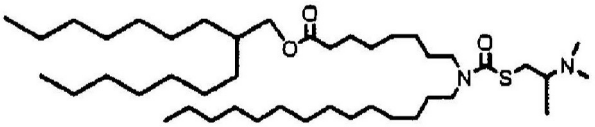
ATX-A-9



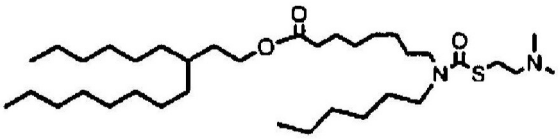
ATX-A-10



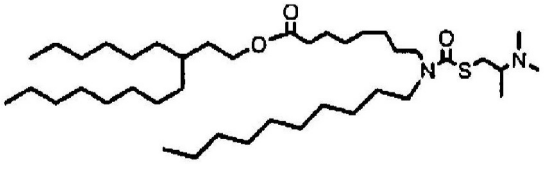
ATX-A-11



ATX-A-12



ATX-A-13



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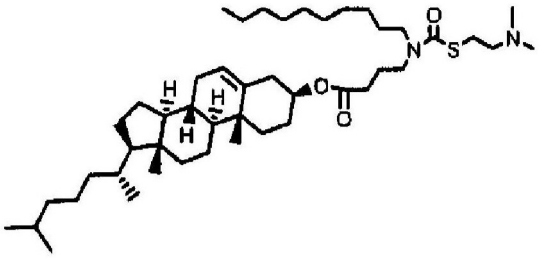
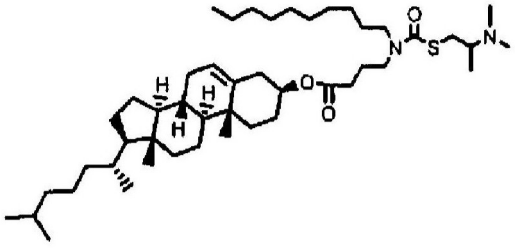
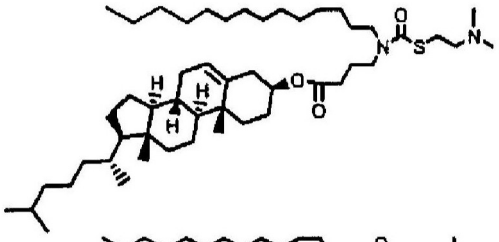


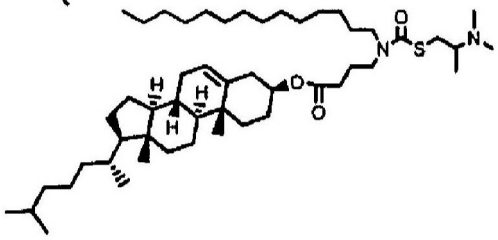
ATX-A-15



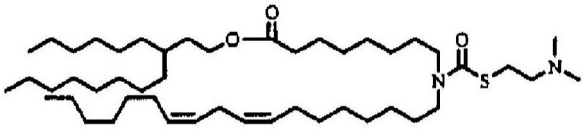
ATX-A-16



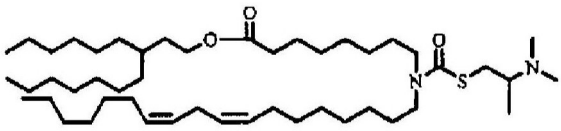
ATX-A-17



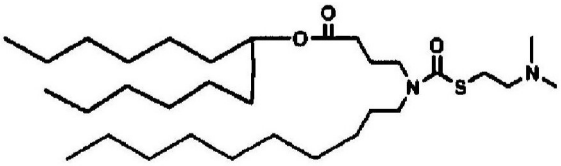
ATX-A-18



ATX-A-19



ATX-A-20



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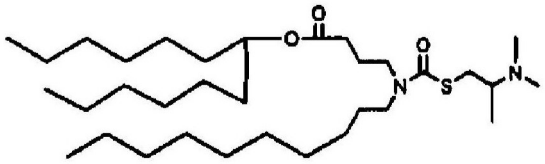

ATX-A-22

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用于RNA递送的可电离阳离子脂质

[0001] 相关申请的交叉参考

[0002] 本申请要求2015年5月8日提交的部分继续申请美国专利申请第14/707,796号和2015年5月8日提交的美国专利申请第14/707,876号以及2014年11月18日提交的美国专利申请第14/546,105号的优先权。

背景技术

[0003] 多种不同类型的核酸目前正被开发成用于治疗多种疾病的治疗剂。这些核酸包括基因疗法中的DNA、质粒类干扰核酸、用于RNA干扰(RNAi)的小干扰核酸,包括siRNA、miRNA、反义分子、核酶和适体。由于正在开发这些分子,因此已出现以如下形式制造这些分子的需要,所述形式稳定、存放期长并且可易于合并到无水有机或无水极性非质子溶剂中,使得能够封装核酸而不存在可能在极性水溶液或非极性溶剂中发生的副反应。

[0004] 本发明涉及新颖的脂质组合物,其有助于生物活性和治疗性分子的细胞内递送。本发明还涉及包含这类脂质组合物并用于将治疗有效量的生物活性分子递送至患者细胞中的医药组合物。

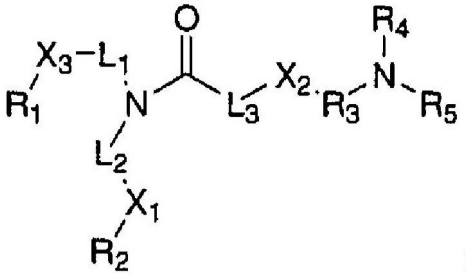
[0005] 治疗性化合物向个体的递送对于其治疗作用是重要的,并且通常可能因所述化合物到达靶细胞和组织的能力有限而受到阻碍。通过各种递送方式改善这类化合物进入组织的靶细胞是至关重要的。本发明涉及有助于生物活性分子的靶向细胞内递送的组合物和制备方法中的新颖脂质。

[0006] 通常未实现有效靶向患者组织的生物活性分子的实例包括:(1)许多蛋白质,包括免疫球蛋白;(2)聚核苷酸,如基因组DNA、cDNA或mRNA;(3)反义聚核苷酸;和(4)许多低分子量化合物,不论是合成或是天然存在的,如肽激素和抗生素。

[0007] 执业医生现在所面对的一个基本挑战是多种不同类型的核酸目前正被开发成用于治疗多种疾病的治疗剂。这些核酸包括基因疗法中的DNA、质粒、小干扰核酸(siNA)、用于RNA干扰(RNAi)的siRNA和微RNA(miRNA)、反义分子、核酶、反义微RNA和适体。由于正在开发这些核酸,因此需要制造容易制备并且可易于递送至目标组织的脂质配制品。

发明内容

[0008] 本文所描述的是式I、II、III、IV和V化合物。所描述的是式I化合物

[0009] 

I

[0010] 其中

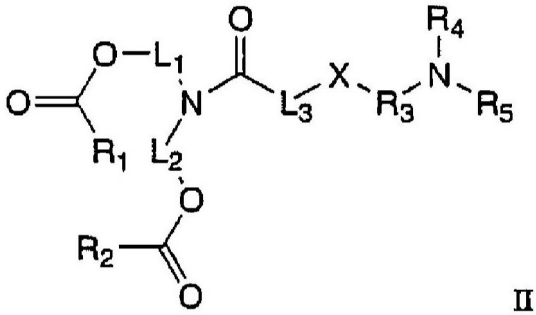
[0011] R_1和R_2各自由1至9个碳所组成的直链烷基、2至11个碳所组成的烯基或炔基组成;

L_1和L_2各自由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；X_1和X_3都由-CO-O-组成；

[0012] X_2由S或O组成；L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；R_3由1至6个碳所组成的直链或支链亚烷基组成；并且R_4和R_5是相同或不同的，各自由氢或1至6个碳所组成的直链或支链烷基组成；

[0013] 或其药学上可接受的盐。

[0014] 本文还描述的是式II化合物

[0015] 

II

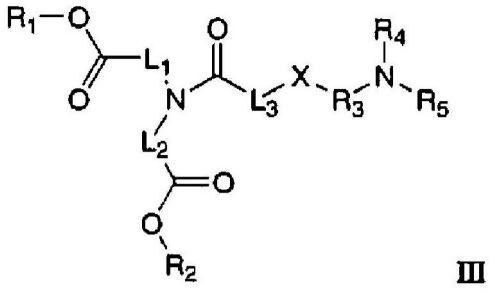
[0016] 其中

[0017] R_1和R_2都由1至12个碳所组成的直链烷基、2至12个碳所组成的烯基或炔基组成；L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；X由S组成；L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；R_3由1至6个碳所组成的直链或支链亚烷基组成；并且R_4和R_5是相同或不同的，各自由氢或1至6个碳所组成的直链或支链烷基组成；

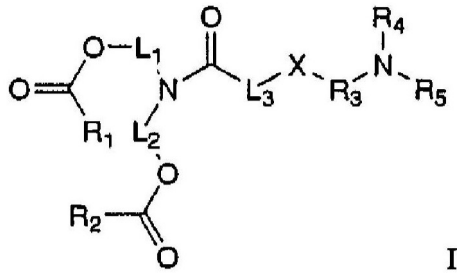
[0018] 或其药学上可接受的盐。

[0019] 在式II化合物的一个实施例中，L_1和L_2都由五个碳所组成的直链亚烷基组成。在式I化合物的另一个实施例中，R_3是亚乙基或亚丙基。在式I化合物的另一个实施例中，R_4和R_5是相同或不同的，各自是氢、甲基或乙基。在式I化合物的另一个实施例中，L_3是键。在式I化合物的另一个实施例中，R_1和R_2都由十个碳所组成的直链烯基组成。

[0020] 本文还描述的是式III或IV化合物

[0021] 

III



IV

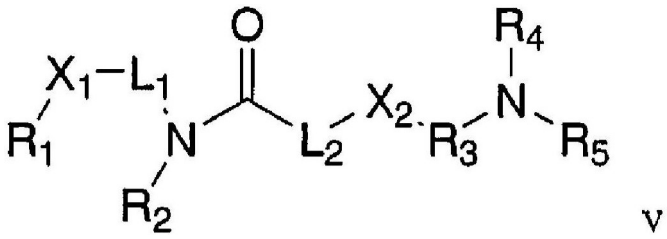
[0022] 其中

[0023] R_1由具有12至20个碳的支链烷基组成;R_2由具有5至10个碳的直链烷基或具有12至20个碳的支链烷基组成;L_1和L_2各自由键或具有1至3个碳原子的直链烷基组成;X由S或O组成;L_3由键或低碳烷基组成;R_3是低碳烷基,以及

[0024] R_4和R_5是相同或不同的,各自由低碳烷基组成;或其药学上可接受的盐。

[0025] 在式III或IV化合物的一个实施例中,L_3由键组成。式III或IV化合物的另一个实施例,X是S。在式III或IV化合物的另一个实施例中,R_3是亚乙基。在式III或IV化合物的另一个实施例中,R_3是亚正丙基或亚异丙基。在式III或IV化合物的另一个实施例中,R_4和R_5分别是甲基、乙基或异丙基。在式III或IV化合物的另一个实施例中,L_1和L_2各自由键组成。在式III或IV化合物的另一个实施例中,L_1和L_2各自由亚甲基组成。在式III或IV化合物的另一个实施例中,R_1和R_2各自由支链烷基组成。在式III或IV化合物的另一个实施例中,R_2由烷基组成。在式III或IV化合物的另一个实施例中,R_1和R_2各自由19或20个碳原子组成。在式III或IV化合物的另一个实施例中,R_1或R_2各自由13或14个碳原子组成。在式III或IV化合物的另一个实施例中,L_3由亚甲基组成,R_3是亚乙基,X_2是S,并且R_4和R_5各自是甲基。在式III或IV化合物的另一个实施例中,其中L_3由键组成,R_3是亚乙基,X是S,并且R_4和R_5各自是甲基。在式III或IV化合物的另一个实施例中,L_3由键组成,R_3由亚正丙基组成,X由S组成,并且R_4和R_5各自由甲基组成。在式III或IV化合物的另一个实施例中,L_3由键组成,R_3由亚异丙基组成,X由S组成,并且R_4和R_5各自由甲基组成。

[0026] 还描述的是式V化合物

[0027] 

V

[0028] 其中

[0029] R_1由1-18个碳所组成的直链或支链烷基、2至12个碳所组成的烯基或炔基、或胆固醇基组成;R_2由1至18个碳所组成的直链或支链烷基或烯基组成;L_1由5至9个碳所组成的直链烷基组成,或当R_1由胆固醇基组成时,那么L_1由3至4个碳所组成的直链亚烷基或烯基组成;X_1由-O-(CO)-或-(CO)-O-组成;X_2由S或O组成;L_2由键或具有1至6个碳的直链亚烷基组成;R_3由具有1至6个碳的直链或支链亚烷基组成;并且R_4和R_5是相同或不同的,各自由1至6个碳的直链或支链烷基组成;

[0030] 或其药学上可接受的盐。

[0031] 在式V化合物的一个实施例中,L_2由键组成。在式V化合物的另一个实施例中,X_2由S组成。在式V化合物的另一个实施例中,X_1是-O-(CO)-。在式V化合物的另一个实施例中,R_3是亚乙基。在式V化合物的另一个实施例中,R_3是亚正丙基或亚异丙基。在式V化合物的另一个实施例中,R_4和R_5分别是甲基、乙基或异丙基。在式V化合物的另一个实施例中,L_2由亚甲基组成。在式V化合物的另一个实施例中,R_1和R_2各自由支链烷基组成。在式V化合物的另一个实施例中,R_2由烷基组成。在式V化合物的另一个实施例中,R_1和R_2各自由19或20个碳原子组成。在式V化合物的另一个实施例中,R_1或R_2各自由13或14个碳原子组成。在式V化合物的另一个实施例中,L_2由亚甲基组成,R_3是亚乙基,X_1是-O-(CO)-,X_2是S,并且R_4和R_5都是甲基。在

式V化合物的另一个实施例中，L_2由键组成，R_3是亚乙基，X_1是-O-(CO)-，X_2是S，并且R_4和R_5都是甲基。在式V化合物的另一个实施例中，L_2由键组成，R_3是亚正丙基，X_1是-O-(CO)-，X_2是S，并且R_4和R_5都是甲基。在式V化合物的另一个实施例中，L_2由键组成，R_3是亚异丙基，X_1是-O-(CO)-，X_1是S，并且R_4和R_5都是甲基。

[0032] 核酸优选地具有抑止目标基因表达的活性。目标基因优选地是与炎症相关联的基因。

[0033] 本文还描述的是一种通过使用以上任一种组合物将核酸引入到哺乳动物细胞中的方法。细胞可在肝脏、肺、肾脏、脑、血液、脾脏或骨骼中。优选地，静脉内、皮下、腹膜内或鞘内投与组合物。优选地，在用于治疗癌症或炎性疾病的方法中使用本文所描述的组合物。疾病可为选自由以下组成的群组的疾病：免疫病症、癌症、肾脏疾病、纤维化疾病、遗传异常、炎症和心血管病症。

附图说明

[0034] 图1示出了由不同阳离子脂质囊封的siRNA的基因敲低活性。脂质包括MC3(0.3mg/kg)、NCI(0.3mg/kg)、ATX-547(0.3mg/kg)、ATX-001(0.3和1.0mg/kg)、ATX-002(0.3和1.0mg/kg)和ATX-003(0.3和1.0mg/kg)。在向C57BL6小鼠投与siRNA配制品之后，示出与仅注射媒剂相比小鼠血浆中基因敲低的因子VII的量。包括异常和正常人类血浆中因子VII的量作为对照。由星号(*)示出因子VII水平的统计学上显著降低($p<0.01$)。

[0035] 图2示出了siRNA对于因子VII活性的作用的评估，其基于图2中所示的结果并且归一化成与仅媒剂相比的基因敲低百分比。

[0036] 图3示出了由不同阳离子脂质囊封的siRNA的基因敲低活性。脂质包括MC3(0.3和1.5mg/kg)、NCl(0.3mg/kg)、AT547(0.1和0.3mg/kg)、AT004(0.3)、AT006(0.3和1.0mg/kg)、ATX-010(0.3mg/kg)和AT001(0.3和1.5mg/kg)。在向C57BL6小鼠投与siRNA配制品之后，示出与仅注射媒剂相比小鼠血浆中基因敲低的因子VII的量。包括异常和正常人类血浆中因子VII的量作为对照。由星号(*)示出因子VII水平的统计学上显著降低($p<0.01$)。

[0037] 图4示出了siRNA对于因子VII活性的作用的评估，其基于图2中所示的结果并且归一化成与仅媒剂相比的基因敲低百分比。

具体实施方式

[0038] “至少一个”意思指一个或多个(例如，1-3、1-2或1个)。

[0039] “组合物”包括包含规定量的规定成分的产物，以及由规定量的规定成分的组合直接或间接产生的任何产物。

[0040] “与.....组合”在用于描述本发明的治疗方法中式1、I和II化合物与其它药剂的投与时，意思指式1、I和II化合物与其它药剂以分开的剂型依次或同时投与，或以同一剂型同时投与。

[0041] “哺乳动物”意思指人类或其它哺乳动物，或意思指人类。

[0042] “患者”包括人类和其它哺乳动物，优选地人类。

[0043] “烷基”意思指饱和或不饱和的直链或支链烃链。在各种实施例中，烷基具有1-18个碳原子，即是C_1-C_{18}基团，或是C_1-C_{12}基团、C_1-C_6基团或C_1-C_4基团。独立地，在各种实施例

中，烷基具有零个分支(即，是直链)、一个分支、两个分支或大于两个分支。“烯基”意思指可具有一个双键、两个双键、大于两个双键的不饱和烷基。“炔基”意思指可具有一个三键、两个三键或大于两个三键的不饱和烷基。烷基链可任选地经1个取代基(即，烷基是经单取代的)、或1-2个取代基、或1-3个取代基、或1-4个取代基取代。取代基可选自由以下组成的群组：羟基、氨基、烷基氨基、硼基、羧基、硝基、氰基或卤基。当烷基并入一个或多个杂原子时，烷基在本文中被称为杂烷基。当烷基上的取代基是烃时，那么所得基团简称为经取代的烷基。在各种方面中，包括取代基的烷基具有小于25、24、23、22、21、20、19、18、17、16、15、14、13、12、11、10、9、8或7个碳。

[0044] “低碳烷基”意思指在链中具有一至六个碳原子的基团，所述链可为直链或支链。合适烷基的非限制性实例包括甲基、乙基、正丙基、异丙基、正丁基、叔丁基、正戊基和己基。

[0045] “烷氧基”意思指烷基-O-基团，其中烷基如上文所定义。烷氧基的非限制性实例包括：甲氧基、乙氧基、正丙氧基、异丙氧基、正丁氧基和庚氧基。经由醚氧键结至母体部分。

[0046] “烷氧基烷基”意思指烷氧基-烷基-基团，其中烷氧基和烷基如先前所描述。优选的烷氧基烷基包含低碳烷基。经由烷基键结至母体部分。

[0047] “烷芳基”意思指烷基-芳基-基团，其中烷基和芳基如先前所描述。优选的烷芳基包含低碳烷基。经由芳基键结至母体部分。

[0048] “氨基烷基”意思指经由烷基键结至母体部分的NH_2-烷基-基团，其中烷基如上文所定义。

[0049] “羧基烷基”意思指经由烷基键结至母体部分的HOOC-烷基-基团，其中烷基如上文所定义。

[0050] “可商购的化学物质”和本文阐述的实例中所用的化学物质可从标准商业来源获得，其中这类来源包括例如Acros Organics(宾夕法尼亚州匹兹堡)、Sigma-Adrich Chemical(威斯康星州密尔沃基)、Avocado Research(英国兰开夏)、Bionet(英国康沃尔)、Boron Molecular(北卡罗莱纳州三角研究园)、Combi-Blocks(加利福尼亚州圣地亚哥)、Eastman Organic Chemicals，Eastman Kodak Company(纽约罗契斯特)、Fisher Scientific Co.(宾夕法尼亚州匹兹堡)、Frontier Scientific(犹他州洛根)、ICN Biomedicals，Inc.(加利福尼亚州科斯塔梅沙)、Lancaster Synthesis(新罕布什尔州温德姆)、Maybridge Chemical Co.(英国康沃尔)、Pierce Chemical Co.(伊利诺斯州罗克福德)、Riedel de Haen(德国汉诺威)、Spectrum Quality Product，Inc.(新泽西州新不伦瑞克)、TCI America(俄勒冈州波特兰)和Wako Chemicals USA，Inc.(弗吉尼亚州里奇蒙)。

[0051] “化学文献中所描述的化合物”可如所属领域的一般技术人员已知经由关于化合物和化学反应的参考书和数据库鉴别。详述用于制备本文所公开的化合物的反应物的合成或对描述本文所公开的化合物的制备的文章提供参考的合适参考书和论文包括例如“合成有机化学(Synthetic Organic Chemistry)”，John Wiley and Sons，Inc.纽约；S.R-Sandler等人，“有机官能团制备(Organic Functional Group Preparations)”第2版，Academic Press，纽约，1983；H.O.House，“现代合成反应(Modern Synthetic Reactions)，”第2版，W.A.Benjamin，Inc.加利福尼亚州门洛帕克，1972；T.L.Glichrist，“杂环化学(Heterocyclic Chemistry)，”第2版.John Wiley and Sons，纽约，1992；J.March，“先进有机化学：反应、机制和结构(Advanced Organic Chemistry:reactions，

Mechanisms and Structure)，”第5版，Wiley Interscience，纽约，2001；特定和类似反应物也可经由美国化学学会的化学文摘社(Chemical Abstract Service of the American Chemical Society)所制备的已知化学物质的索引来鉴别，所述索引可用于大部分公共图书馆和大学图书馆以及遍及在线数据库(例如华盛顿的美国化学学会)。已知但目录中不可商购的化学物质可由定制化学合成室制备，其中许多标准化学供应室(如上文所列的那些)提供定制合成服务。

[0052] “卤基”意思指氟基、氯基、溴基或碘基。优选的是氟基、氯基或溴基，并且更优选的是氟基和氯基。

[0053] “卤素”意思指氟、氯、溴或碘。优选的是氟、氯和溴。

[0054] “杂烷基”是含有碳和至少一个杂原子的饱和或不饱和直链或支链。在各种实施例中，杂烷基可具有一个杂原子、或1-2个杂原子、或1-3个杂原子、或1-4个杂原子。在一个方面，杂烷基链含有1至18(即，1-18)个成员原子(碳和杂原子)，并且在各种实施例中含有1-12、或1-6、或1-4个成员原子。独立地，在各种实施例中，杂烷基具有零个分支(即，是直链)、一个分支、两个分支或大于两个分支。独立地，在一个实施例中，杂烷基是饱和的。在另一个实施例中，杂烷基是不饱和的。在各种实施例中，不饱和杂烷基可具有一个双键、两个双键、大于两个双键，和/或一个三键、两个三键或大于两个三键。杂烷基链可经取代或未经取代。在一个实施例中，杂烷基链未经取代。在另一个实施例中，杂烷基链经取代。经取代的杂烷基链可具有1个取代基(即，通过单取代)，或可具有例如1-2个取代基、或1-3个取代基、或1-4个取代基。示例性杂烷基取代基包括酯(-C(O)-O-R)和羧基(-C(O)-)。

[0055] “羟基烷基”意思指HO-烷基-基团，其中烷基如先前所定义。优选的羟基烷基含有低碳烷基。合适的羟基烷基的非限制性实例包括羟甲基和2-羟乙基。

[0056] “水合物”是其中溶剂分子是H_2O的溶剂合物。

[0057] “脂质”意思指包含脂肪酸酯并且特征在于不可溶于水、但可溶于许多有机溶剂中的有机化合物。脂质通常分成至少三类：(1)“简单脂质”，其包括脂肪和油以及蜡；(2)“化合物脂质”，其包括磷脂和糖脂；和(3)“衍生脂质”，如类固醇。

[0058] “脂质粒子”意思指可用于递送治疗性核酸(例如，mRNA)至所关注的目标位点(例如，细胞、组织、器官等等)的脂质配制品。在优选实施例中，脂质粒子是核酸-脂质粒子，其通常由阳离子脂质、非阳离子脂质(例如，磷脂)、防止粒子聚集的结合脂质(例如，PEG-脂质)和任选地胆固醇形成。通常，治疗性核酸(例如，mRNA)可囊封于粒子的脂质部分中，从而保护其免于酶降解。

[0059] 脂质粒子的平均直径通常是30nm至150nm、40nm至150nm、50nm至150nm、60nm至130nm、70nm至110nm、70nm至100nm、80nm至100nm、90nm至100nm、70至90nm、80nm至90nm、70nm至80nm、或30nm、35nm、40nm、45nm、50nm、55nm、60nm、65nm、70nm、75nm、80nm、85nm、90nm、95nm、100nm、105nm、110nm、115nm、120nm、125nm、130nm、135nm、140nm、145nm或150nm，并且基本上是无毒的。另外，核酸，当存在于本发明的脂质粒子中时，在水溶液中抗核酸酶降解。

[0060] “溶剂合物”意思指本发明化合物与一个或多个溶剂分子的物理性缔合。此物理性缔合涉及不同程度的离子和共价键结，包括氢键结。在某些情况下，溶剂合物将能够分离，例如当一个或多个溶剂分子并入结晶固体的晶格中时。“溶剂合物”涵盖溶液相和可分离的

溶剂合物。合适溶剂合物的非限制性实例包括乙醇合物、甲醇合物等等。

[0061] “经脂质囊封”可意思指提供完全囊封、部分囊封或兼具的治疗性核酸(如mRNA)的脂质粒子。在一优选实施例中，核酸(例如，mRNA)完全囊封于脂质粒子中。

[0062] “脂质结合物”意思指抑制脂质粒子聚集的结合脂质。这类脂质结合物包括(但不限于)PEG-脂质结合物，如偶合到二烷氧基丙基的PEG(例如，PEG-DAA结合物)、偶合到二酰基甘油的PEG(例如，PEG-DAG结合物)、偶合到胆固醇的PEG、偶合到磷脂酰乙醇胺的PEG和结合到神经酰胺的PEG；阳离子PEG脂质；聚噁唑啉(POZ)-脂质结合物；聚酰胺寡聚物(例如，ATTA-脂质结合物)和其混合物。PEG或POZ可直接结合到脂质或可经由连接体部分连接到脂质。可使用适用于将PEG或POZ偶合到脂质的任何连接体部分，包括例如不含酯的连接体部分和含有酯的连接体部分。在某些优选实施例中，使用不含酯的连接体部分，如酰胺或氨基甲酸酯。

[0063] “两性脂质”意思指其中脂质物质的疏水性部分定向于疏水相，而亲水性部分定向于水相的物质。亲水性特征源自存在极性或带电荷基团，如碳水化合物、磷酸酯基、羧基、硫酸根合、氨基、巯基、硝基、羟基和其它类似基团。疏水性可通过包括非极性基团而赋予，非极性基团包括(但不限于)长链饱和与不饱和脂族烃基并且这类基团经一个或多个芳香族基团、环脂族基团或杂环基团取代。两性化合物的实例包括(但不限于)磷脂、氨基脂和鞘脂。

[0064] 磷脂的代表性实例包括(但不限于)磷脂酰胆碱、磷脂酰乙醇胺、磷脂酰丝氨酸、磷脂酰肌醇、磷脂酸、棕榈酰油酰基磷脂酰胆碱、溶血磷脂酰胆碱、溶血磷脂酰乙醇胺、二棕榈酰基磷脂酰胆碱、二油酰基磷脂酰胆碱、二硬脂酰基磷脂酰胆碱和二亚油酰基磷脂酰胆碱。缺少磷的其它化合物，如鞘脂、鞘糖脂家族、二酰基甘油和β-酰氧基酸，也在称为两性脂质的群组内。此外，上文所描述的两性脂质可与包括甘油三酯和固醇的其它脂质混合。

[0065] “中性脂质”意思指在所选pH下以不带电荷或中性两性离子形式存在的脂质种类。在生理pH下，这类脂质包括例如二酰基磷脂酰胆碱、二酰基磷脂酰乙醇胺、神经酰胺、鞘磷脂、脑磷脂、胆固醇、脑苷脂和二酰基甘油。

[0066] “非阳离子脂质”意思指两性脂质或中性脂质或阴离子脂质，并且更详细地描述于下文。

[0067] “阴离子脂质”意思指在生理pH下带负电荷的脂质。这些脂质包括(但不限于)磷脂酰甘油、心肌磷脂、二酰基磷脂酰丝氨酸、二酰基磷脂酸、N-十二酰基磷脂酰乙醇胺、N-丁二酰基磷脂酰乙醇胺、N-戊二酰基磷脂酰乙醇胺、赖氨酰基磷脂酰甘油、棕榈酰油酰基磷脂酰甘油(POPG)和其它阴离子修饰基团接合到中性脂质。

[0068] 术语“疏水性脂质”意思指具有非极性基团的化合物，非极性基团包括(但不限于)长链饱和与不饱和脂族烃基并且这类基团任选地经一个或多个芳香族基团、环脂族基团或杂环基团取代。合适实例包括(但不限于)二酰基甘油、二烷基甘油、N-N-二烷基氨基、1，2-二酰氧基-3-氨基丙烷和1，2-二烷基-3-氨基丙烷。

[0069] 术语“阳离子脂质”和“氨基脂”在本文中可互换用于包括那些具有一个、两个、三个或更多个脂肪酸或脂肪烷基链和pH可滴定的氨基头部基团(例如，烷基氨基或二烷基氨基头部基团)的脂质和其盐。阳离子脂质通常在低于阳离子脂质pK_a的pH下质子化(即，带正电荷)并且在高于pK_a的pH下基本上是中性的。本发明的阳离子脂质也可称为可滴定的阳离

子脂质。在一些实施例中，阳离子脂质包含：可质子化叔胺(例如，pH可滴定)头部基团；C_{18}烷基链，其中每一烷基链独立地具有0至3(例如，0、1、2或3)个双键；和头部基团与烷基链之间的醚、酯或缩酮键。这类阳离子脂质包括(但不限于)DSDMA、DODMA、DLinDMA、DLenDMA、γ-DLenDMA、DLin-K-DMA、DLin-K-C2-DMA(也称为DLin-C2K-DMA、XTC2和C2K)、DLin-K-C3-DMA、DLin-K-C4-DMA、DLen-C2K-DMA、y-DLen-C2K-DMA、DLin-M-C2-DMA(也称为MC2)、DLin-M-C3-DMA(也称为MC3)和(DLin-MP-DMA)(也称为1-B11)。

[0070] 术语“经取代”意思指经除氢以外的规定基团或经一个或多个可能相同或不同的基团、部分或自由基(其中每一个例如是独立选择的)取代。

[0071] “反义核酸”意思指借助于RNA-RNA或RNA-DNA或RNA-PNA(蛋白质核酸；Egholm等人，1993自然(Nature)365，566)相互作用结合于目标RNA并且改变目标RNA的活性的非酶核酸分子(关于综述，参见Stein和Cheng，1993科学(Science)261，1004和Woolf等人，美国专利第5,849,902号)。通常，反义分子沿着反义分子的单个连续序列与目标序列互补。然而，在某些实施例中，反义分子可结合于底物以使得底物分子形成环，和/或反义分子可结合以使得反义分子形成环。因此，反义分子可与两个(或甚至更多)不连续底物序列互补，或反义分子的两个(或甚至更多)不连续序列部分可与目标序列互补，或两者都有。另外，反义DNA可借助于DNA-RNA相互作用而用于靶向RNA，从而活化RNA酶H，分解双螺旋体形式的目标RNA。反义寡核苷酸可包含一个或多个RNA酶H活化区，其能够活化RNA酶H裂解目标RNA。反义DNA可以化学方式合成或经由使用单链DNA表达载体或其等效物表达。反义RNA是序列与目标基因mRNA互补的RNA链。有义RNA具有与反义RNA互补的序列，并且粘接至其互补反义RNA以形成iNA。这些反义和有义RNA已用RNA合成仪常规合成。

[0072] “核酸”是指呈单链或双链形式的脱氧核糖核苷酸或核糖核苷酸和其聚合物。所述术语涵盖含有已知核苷酸类似物或经修饰的主链残基或键的核酸，其是合成、天然存在和非天然存在的，具有与参考核酸类似的结合特性，并且以与参考核苷酸类似的方式代谢。这类类似物的实例包括(但不限于)硫代磷酸酯、氨基磷酸酯、膦酸甲酯、手性膦酸甲酯、2′-O-甲基核糖核苷酸、肽-核酸(PNA)。

[0073] “RNA”意思指包含至少一个核糖核苷酸残基的分子。“核糖核苷酸”意思指在β-D-呋喃核糖部分的2′位置具有羟基的核苷酸。所述术语包括双链RNA、单链RNA、经分离的RNA(如部分纯化的RNA)、基本上纯的RNA、合成RNA、重组产生的RNA以及通过添加、缺失、取代和/或改变一个或多个核苷酸而与天然存在的RNA不同的经改变的RNA。这类改变可包括如向干扰RNA的端部或内部(例如在RNA的一个或多个核苷酸处)添加非核苷酸物质。本发明的RNA分子中的核苷酸还可包含非标准核苷酸，如非天然存在的核苷酸或化学合成的核苷酸或脱氧核苷酸。这些经改变的RNA可称为类似物或天然存在的RNA的类似物。如本文所用，术语“核糖核酸”和“RNA”是指含有至少一个核糖核苷酸残基的分子，包括siRNA、反义RNA、单链RNA、微RNA、mRNA、非编码RNA和多价RNA。核糖核苷酸是在β-D-呋喃核糖部分的2′位置具有羟基的核苷酸。这些术语包括双链RNA(dsRNA)、单链RNA(ssRNA)、经分离的RNA(如部分纯化的RNA)、基本上纯的RNA、合成RNA、重组产生的RNA以及通过添加、缺失、取代、修饰和/或改变一个或多个核苷酸而与天然存在的RNA不同的经修饰和改变的RNA。RNA的改变可包括如向干扰RNA的端部或内部(例如在RNA的一个或多个核苷酸处)添加非核苷酸物质，RNA分子中的核苷酸包括非标准核苷酸，如非天然存在的核苷酸或化学合成的核苷酸或脱氧核苷

酸。这些经改变的RNA可称为类似物。

[0074] 如本文所用,“核苷酸”如所属领域所公认包括天然碱基(标准)和所属领域中众所周知的修饰碱基。这类碱基一般位于核苷酸糖部分的1′位置。核苷酸一般包含碱基、糖和磷酸基。核苷酸可未经修饰或在糖、磷酸和/或碱基部分处经修饰(还可互换地称为核苷酸类似物、经修饰的核苷酸、非天然核苷酸、非标准核苷酸和其它;参见例如Usman和McSwiggen,见上文;Eckstein等人,国际PCT公开案第WO 92/07065号;Usman等人,国际PCT公开案第WO 93/15187号;Uhlman和Peyman,见上文,全部特此以引用的方式并入本文中)。存在所属领域中已知经修饰的核酸碱基的数种实例,如由Limbach等人,核酸研究(Nucleic Acids Res.)22:2183,1994所汇总。可引入到核酸分子中的碱基修饰的一些非限制性实例包括:肌苷、嘌呤、吡啶-4-酮、吡啶-2-酮、苯基、假尿嘧啶、2,4,6-三甲氧基苯、3-甲基尿嘧啶、二氢尿苷、萘基、氨基苯基、5-烷基胞苷(例如,5-甲基胞苷)、5-烷基尿苷(例如,核糖胸苷)、5-卤尿苷(例如,5-溴尿苷)或6-氮杂嘧啶或6-烷基嘧啶(例如,6-甲基尿苷)、丙炔等(Burgin等人,生物化学(Biochemistry)35:14090,1996;Uhlman和Peyman,见上文)。在此方面中,“经修饰的碱基”意思指在1′位置处的除腺嘌呤、鸟嘌呤、胞嘧啶和尿嘧啶以外的核苷酸碱基或其等效物。

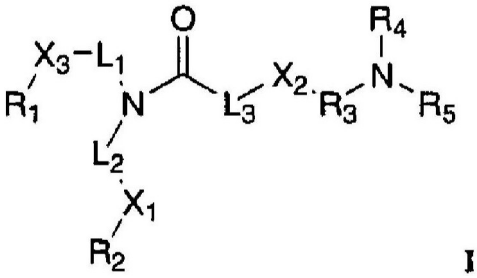
[0075] 如本文所用,互补核苷酸碱基是彼此形成氢键的一对核苷酸碱基。腺嘌呤(A)与胸腺嘧啶(T)或与RNA中的尿嘧啶(U)配对,并且鸟嘌呤(G)与胞嘧啶(C)配对。核酸的互补区段或链彼此杂交(通过氢键结接合)。“互补”意思指核酸可通过传统沃森-克里克(Watson-Crick)结合模式或通过其它非传统结合模式与另一核酸序列形成氢键。

[0076] 微RNA(miRNA)是调节基因表达的21-23个核苷酸长的单链RNA分子,miRNA是由从DNA转录但不翻译成蛋白质的基因编码(非编码RNA);实际上,其是由称为原miRNA的初级转录物加工成称为前miRNA的短茎-环结构并且最后加工成功能性miRNA。成熟miRNA分子与一个或多个信使RNA(mRNA)分子部分互补,并且其主要功能是下调基因表达

[0077] 如本文所用,术语“小干扰RNA(siRNA)”,有时称为短干扰RNA或沉默RNA,用以指一类16-40个核苷酸长的dsRNA分子,其在生物学中发挥多种作用。最值得注意的是,siRNA参与RNA干扰(RNAi)途径,其中其干扰特定基因的表达。除了在RNAi途径中的作用以外,siRNAs还在RNAi相关途径中起作用,例如充当抗病毒机制或用于塑造基因组的染色质结构;现在仅阐明这些途径的复杂性。

[0078] 如本文所用,术语RNAi是指受RNA诱导的沉默复合物(RISC)控制并且由细胞中的短dsRNA分子引发的RNA依赖性基因沉默过程,其中短dsRNA分子与称为argonaute蛋白的催化性RISC组分相互作用。当dsRNA或RNA样iNA或siRNA是外源性(来自受具有RNA基因组的病毒感染或来自转染iNA或siRNA)时,RNA或iNA被直接导入到细胞质中并且由称为Dicer的酶裂解成短片段。启动dsRNA也可为内源性(起源于细胞中),如在由基因组中的RNA编码基因所表达的前miRNA中。这类基因的初级转录物首先加工以形成细胞核中前miRNA的特征性茎-环结构,随后导出至细胞质以待由Dicer裂解。因此,两个dsRNA途径(外源性和内源性)在RISC复合物处会聚。RISC的活性组分argonaute蛋白裂解与其结合的siRNA或iNA互补的目标mRNA链。因为由Dicer产生的片段是双链,所以其可能各自在理论上产生功能性siRNA或iNA。然而,两条链中仅称为引导链的一条链结合argonaute蛋白并且指导基因沉默。另一条反引导链或过客链在RISC活化期间降解。

[0079] 本文所描述的是式I、II、III、IV和V化合物。本文所描述的是式I化合物

[0080] 

I

[0081] 其中

[0082] R_1和R_2都由1至9个碳所组成的直链烷基、2至11个碳所组成的烯基或炔基组成；

[0083] L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；

[0084] X_1和X_3都由-CO-O-组成；

[0085] X_2是S或O；

[0086] L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；

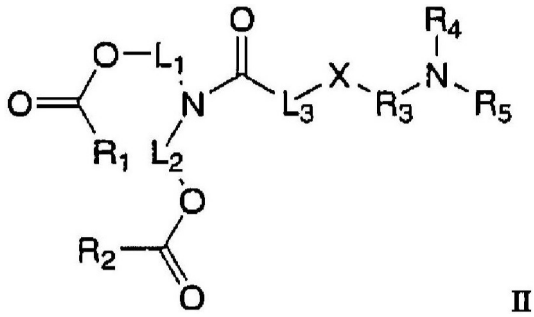
[0087] R_3由1至6个碳所组成的直链或支链亚烷基组成；以及

[0088] R_4和R_5是相同或不同的，由氢或1至6个碳所组成的直链或支链烷基组成，

[0089] 或其药学上可接受的盐。

[0090] 本文还描述的是下表1中列出的ATX-001至ATX-017、ATX-021至ATX-023和ATX-026至ATX-030中列出的任何化合物或其药学上可接受的盐。

[0091] 本文还描述的是式II化合物

[0092] 

II

[0093] 其中

[0094] R_1和R_2都由1至12个碳所组成的直链烷基、2至12个碳所组成的烯基或炔基组成，

[0095] L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环，

[0096] X是S，

[0097] L_3是键或1至6个碳所组成的直链亚烷基，或与N一起形成杂环，

[0098] R_3是1至6个碳所组成的直链或支链亚烷基，以及

[0099] R_4和R_5是相同或不同的，各自是氢或1至6个碳所组成的直链或支链烷基；或药学上可接受的盐。

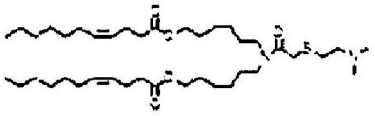
[0100] 在式II化合物的一个实施例中，L_1和L_2都由五个碳所组成的直链亚烷基组成。在式I化合物的另一个实施例中，R_3是亚乙基或亚丙基。在式I化合物的另一个实施例中，R_4和R_5是相同或不同的，各自是氢、甲基或乙基。在式I化合物的另一个实施例中，L_3是键。在式I化合物的另一个实施例中，R_1和R_2都由十个碳所组成的直链烯基组成。

[0101] 在式I和II化合物的另一个实施例中，化合物由选自下表1中列出的ATX-001至

ATX-017、ATX-021至ATX-023和ATX-026至ATX-030中列出的任何化合物、或式1)至12)或其药学上可接受的盐的化合物组成。

[0102]

1)



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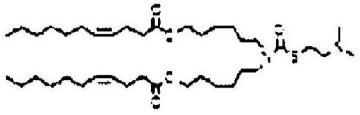

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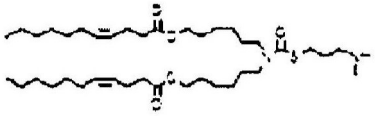

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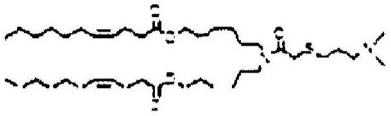

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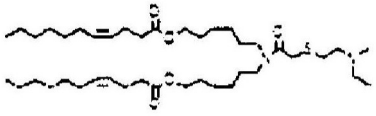

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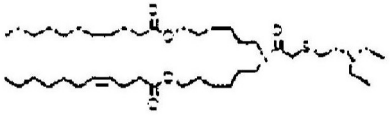

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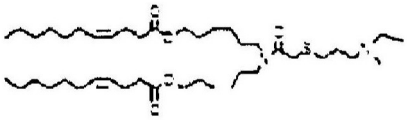

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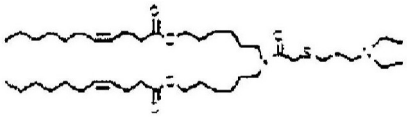

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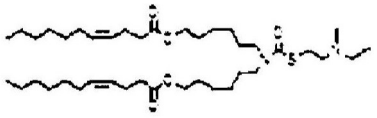

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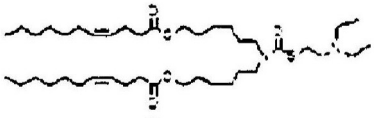

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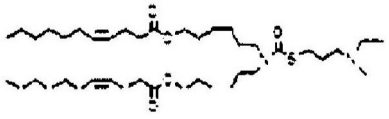

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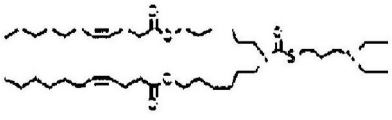
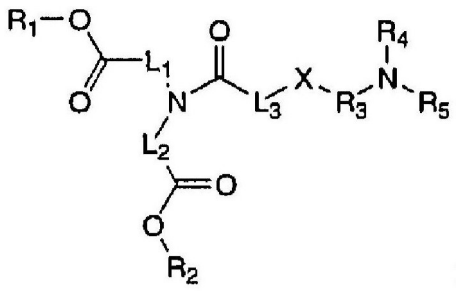
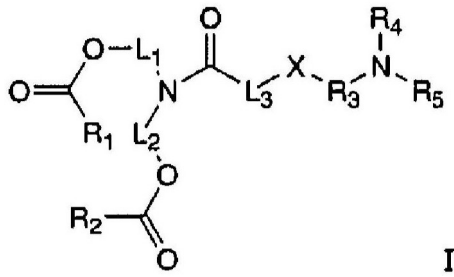


[0103] 本文还描述的是式III或IV化合物

[0104] 

III



IV

[0105] 其中

[0106] R_1由具有12至20个碳的支链烷基组成，

[0107] R_2由具有5至10个碳的直链烷基或具有12至20个碳的支链烷基组成，

[0108] L_1和L_2各自由键或具有1至3个碳原子的直链烷基组成，

[0109] X由S或O组成，

[0110] L_3由键或低碳烷基组成，

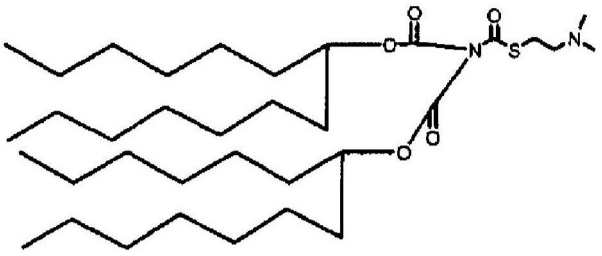
[0111] R_3由低碳烷基组成，以及

[0112] R_4和R_5是相同或不同的，各自由低碳烷基组成；

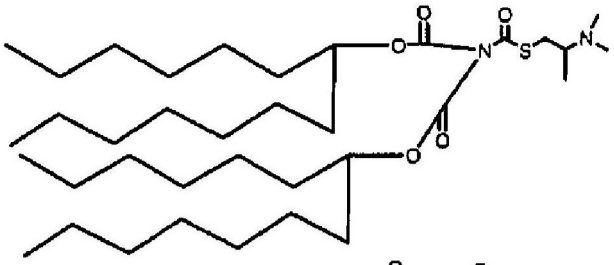
[0113] 或其药学上可接受的盐。

[0114] 在式III或IV化合物的一个实施例中，L_3由键组成。式III或IV化合物的另一个实施例，X是S。在式III或IV化合物的另一个实施例中，R_3是亚乙基。在式III或IV化合物的另一个实施例中，R_3是亚正丙基或亚异丙基。在式III或IV化合物的另一个实施例中，R_4和R_5分别是甲基、乙基或异丙基。在式III或IV化合物的另一个实施例中，L_1和L_2都由键组成。在式III或IV化合物的另一个实施例中，L_1和L_2都由亚甲基组成。在式III或IV化合物的另一个实施例中，R_1和R_2都由支链烷基组成。在式III或IV化合物的另一个实施例中，R_2由烷基组成。在式III或IV化合物的另一个实施例中，R_1和R_2都由19或20个碳原子组成。在式III或IV化合物的另一个实施例中，R_1和R_2都由13或14个碳原子组成。在式III或IV化合物的另一个实施例中，L_3由亚甲基组成，R_3由亚乙基组成，X由S组成，并且R_4和R_5都由甲基组成。在式III或IV化合物的另一个实施例中，其中L_3由键组成，R_3由亚乙基组成，X由S组成，并且R_4和R_5都由甲基组成。在式III或IV化合物的另一个实施例中，L_3由键组成，R_3由亚正丙基组成，X由S组成，并且R_4和R_5都由甲基组成。在式III或IV化合物的另一个实施例中，L_3由键组成，R_3由亚异丙基组成，X由S组成，并且R_4和R_5都由甲基组成。在式III或IV化合物的另一个实施例中，选自由如下式ATX-B-1至ATX-B-12化合物组成的群组。

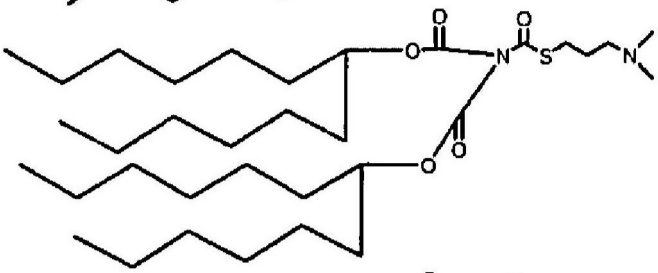
[0115]



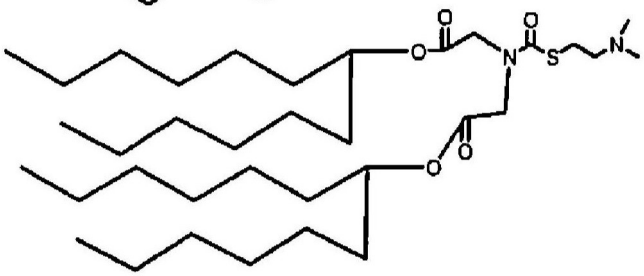
ATX-B-1



ATX-B-2

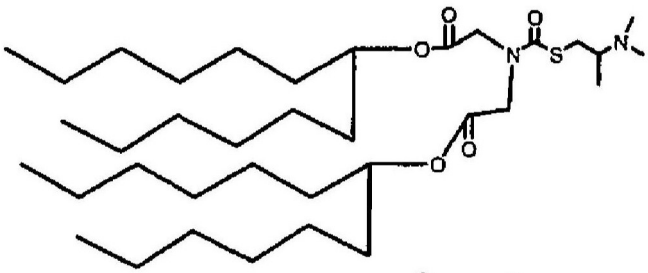


ATX-B-3



ATX-B-4

[0116]



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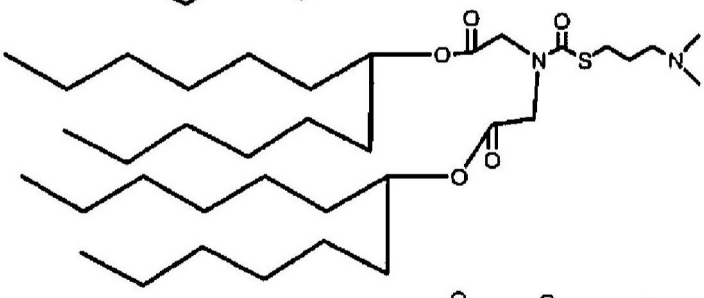

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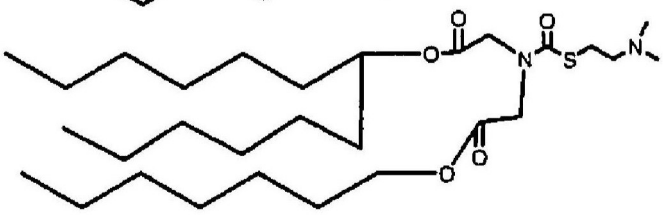

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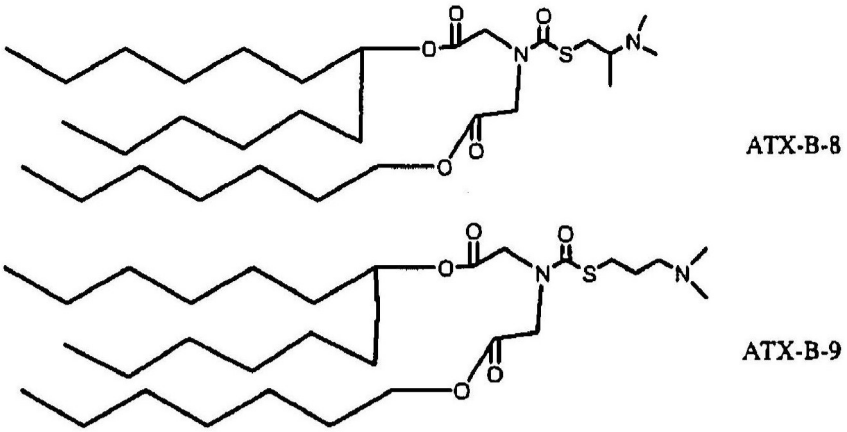
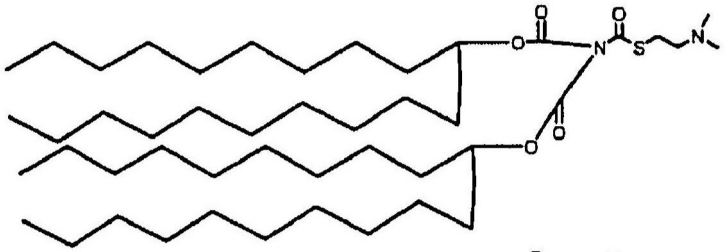


ATX-B-8

ATX-B-9

[0117]



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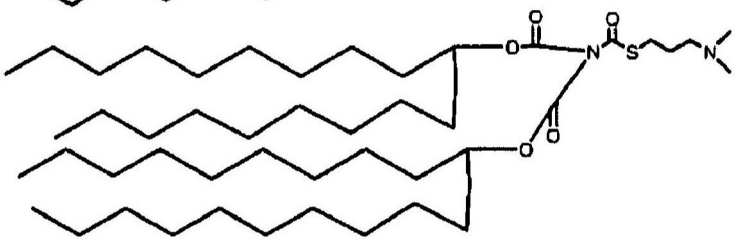
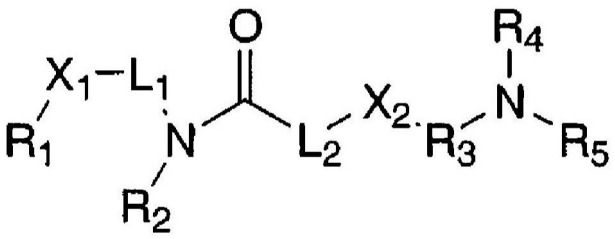


ATX-B-12

[0118] 还描述的是式V化合物

[0119]



V

[0120] 其中

[0121] R_1由1-18个碳所组成的直链或支链烷基、2至12个碳所组成的烯基或炔基、或胆固醇基组成；

[0122] R_2由1至18个碳所组成的直链或支链烷基或烯基组成；

[0123] L_1由5至9个碳所组成的直链烷基组成，或当R_1由胆固醇基组成时，那么L_1由3至4个碳所组成的直链亚烷基或烯基组成；X_1由-O-(CO)-或-(CO)-O-组成；

[0124] X_2由S或O组成；

[0125] L_2由键或具有1至6个碳的直链亚烷基组成；

[0126] R_3由具有1至6个碳的直链或支链亚烷基组成；以及

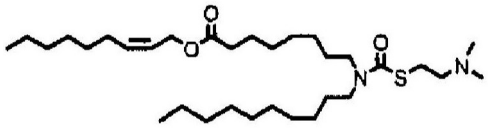
[0127] R_4和R_5是相同或不同的，各自由具有1至6个碳的直链或支链烷基组成；

[0128] 或其药学上可接受的盐

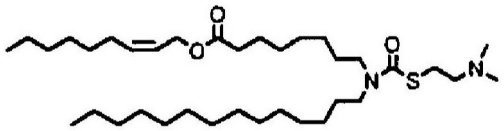
[0129] 在式V化合物的一个实施例中，L_2由键组成。在式V化合物的另一个实施例中，X_2由S组成。在式V化合物的另一个实施例中，X_1是-O-(CO)-。在式V化合物的另一个实施例中，R_3由

亚乙基组成。在式V化合物的另一个实施例中，R_3由亚正丙基或亚异丙基组成。在式V化合物的另一个实施例中，R_4和R_5各自由甲基、乙基或异丙基组成。在式V化合物的另一个实施例中，L_2由亚甲基组成。在式V化合物的另一个实施例中，R_1和R_2各自由支链烷基组成。在式V化合物的另一个实施例中，R_2由烷基组成。在式V化合物的另一个实施例中，R_1和R_2各自由19或20个碳原子组成。在式V化合物的另一个实施例中，R_1或R_2各自由13或14个碳原子组成。在式V化合物的另一个实施例中，L_2由亚甲基组成，R_3由亚乙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。在式V化合物的另一个实施例中，L_2由键组成，R_3是亚乙基，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。在式V化合物的另一个实施例中，L_2由键组成，R_3由亚正丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。在式V化合物的另一个实施例中，L_2由键组成，R_3由亚异丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。在式V化合物的另一个实施例中，化合物是选自式ATX-A-1至ATX-A-22的化合物

[0130]



ATX-A-1



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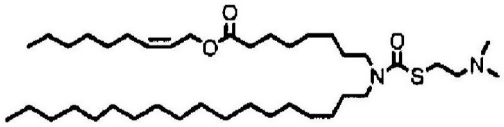
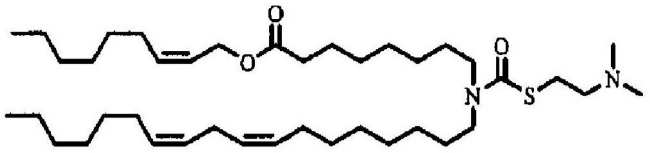


ATX-A-3

[0131]



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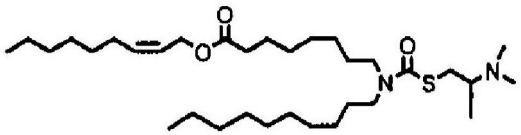

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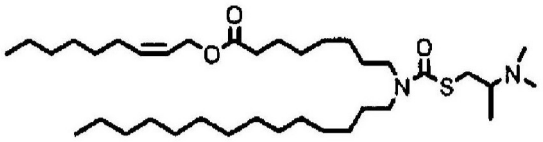

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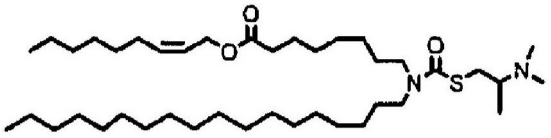

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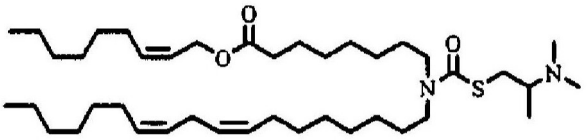

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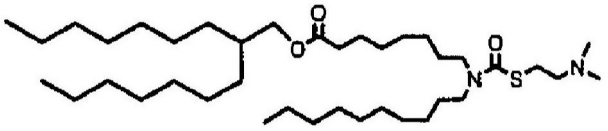

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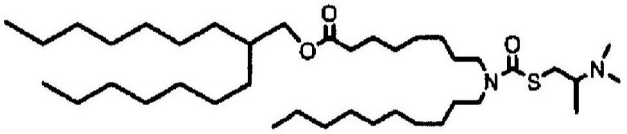

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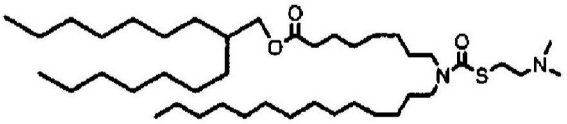

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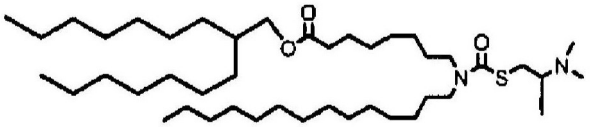

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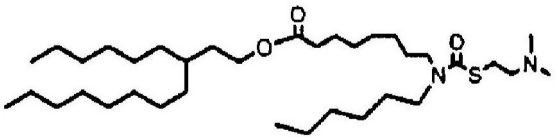

ATX-A-13

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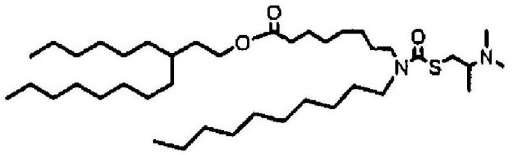

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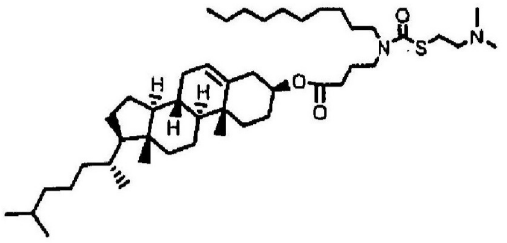

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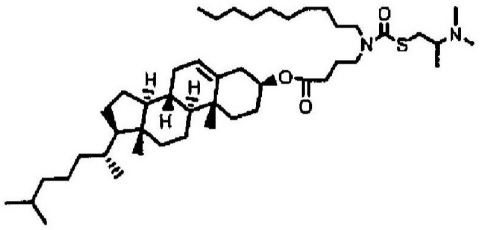

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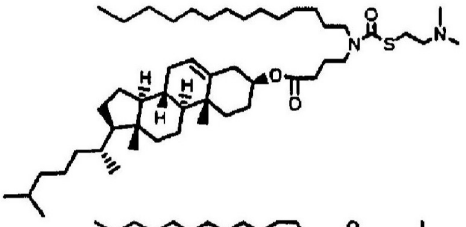
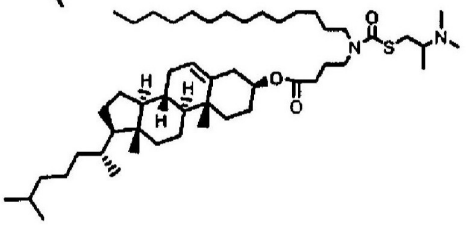
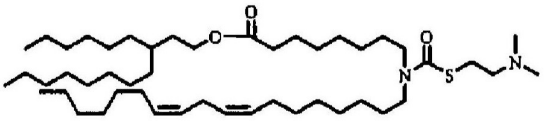


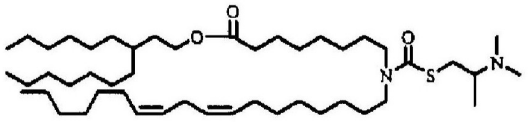
ATX-A-17



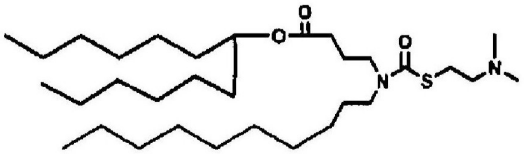
ATX-A-18



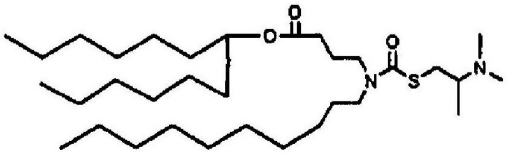
ATX-A-19



ATX-A-20



ATX-A-21



ATX-A-22

[0133] 式I、II、III、IV和V化合物可为其药学上可接受的盐，在包含纳米粒子的脂质组合物或双层脂质分子中。脂质双层优选地另外包含中性脂质或聚合物。脂质组合物优选地包

含液体介质。所述组合物优选地进一步囊封核酸。所述核酸优选地具有通过利用RNA干扰(RNAi)抑止目标基因表达的活性。脂质组合物优选地另外包含核酸和中性脂质或聚合物。脂质组合物优选地囊封核酸。

[0134] 式I、II、III、IV和V化合物形成也在本发明范围内的盐。除非另外指明，否则在本文中提及式I、II、III、IV或V化合物理解为包括提及其盐。如本文所用的术语“盐”表示用无机和/或有机酸形成的酸式盐以及用无机和/或有机碱形成的碱式盐。另外，式I、II、III、IV或V化合物的这类盐可含有碱性部分，如(但不限于)吡啶或咪唑；或酸性部分，如(但不限于)羧酸；和两性离子(“内盐”)。盐可为药学上可接受的(即，无毒性、生理学上可接受的)盐，但是其它盐也是有用的。式I、II、III、IV或V化合物的盐可例如通过使式I、II、III、IV或V化合物与一定量(如等量)的酸或碱在介质(如其中盐沉淀的介质)中或在水性介质中反应，接着冷冻干燥来形成。

[0135] 示例性酸加成盐包括乙酸盐、己二酸盐、海藻酸盐、抗坏血酸盐、天冬氨酸盐、苯甲酸盐、苯磺酸盐、硫酸氢盐、硼酸盐、丁酸盐、柠檬酸盐、樟脑酸盐、樟脑磺酸盐、环戊烷丙酸盐、二葡糖酸盐、十二烷基硫酸盐、乙烷磺酸盐、反丁烯二酸盐、葡庚酸盐、甘油磷酸盐、半硫酸盐、庚酸盐、己酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2-羟基乙烷磺酸盐、乳酸盐、马来酸盐、甲烷磺酸盐、2-萘磺酸盐、烟酸盐、硝酸盐、乙二酸盐、果胶酸盐、过硫酸盐、3-苯丙酸盐、磷酸盐、苦味酸盐、特戊酸盐、丙酸盐、水杨酸盐、丁二酸盐、硫酸盐、磺酸盐(如本文中所提及的那些)、酒石酸盐、硫氰酸盐、甲苯磺酸盐(toluenesulfonate/tosylate)十一烷酸盐等等。此外，一般视为适用于由碱性医药化合物形成药学上有用的盐的酸例如由S.Berge等人，药物科学杂志(J.Pharmaceutical Sciences)(1977)66(1)1-19；P.Gould，国际药剂学杂志(International J.Pharmaceutics)(1986)33201-217；Anderson等人，医药化学的实践(The Practice of Medicinal Chemistry)(1996)，纽约学术出版社(Academic Press，New York)；以及橘皮书(在华盛顿食品药品监督管理局网站上)中论述。这些公开内容以引用的方式并入本文中。

[0136] 示例性碱式盐包括铵盐；碱金属盐，如钠盐、锂盐和钾盐；碱土金属盐，如钙盐和镁盐；具有有机碱(例如，有机胺)的盐，如苯乍生(benzathine)、二环己基胺、哈胺(hydrabamine)(用N,N-双(去氢松香基)乙二胺形成)、N-甲基-D-葡糖胺、N-甲基-D-葡糖酰胺、叔丁胺；和具有如精氨酸或赖氨酸等氨基酸的盐。碱性含氮基团可用如低碳烷基卤化物(例如，甲基、乙基、丙基以及丁基氯化物、溴化物和碘化物)、硫酸二烷基酯(例如，硫酸二甲基、二乙基、二丁基和二戊基酯)、长链卤化物(例如，癸基、月桂基、肉豆蔻基和硬脂基氯化物、溴化物和碘化物)、芳基烷基卤化物(例如，苯甲基和苯乙基溴化物)等试剂季铵化。

[0137] 所有这类酸盐和碱盐预期是在公开内容范围内的药学上可接受的盐，并且出于本公开内容的目的，所有酸盐和碱盐被视为等效于相应式I化合物的游离形式。

[0138] 式I、II、III、IV和V化合物可以非溶剂化和溶剂化形式(包括水合形式)存在。一般来说，出于本公开内容的目的，具有药学上可接受的溶剂(如水、乙醇等等)的溶剂化形式等效于非溶剂化形式。

[0139] 式I、II、III、IV和V化合物和其盐、溶剂合物可以其互变异构形式(例如，呈酰胺或亚氨醚形式)存在。所有这类互变异构形式都作为本公开内容的一部分涵盖在本文中。

[0140] 本公开内容的化合物的多晶型物也在本公开内容的范围内(即，式I化合物的多晶

型物在本公开内容的范围内)。

[0141] 本发明化合物(包括化合物的那些盐、溶剂合物和前药以及前药的盐和溶剂合物)的所有立体异构体(例如,几何异构体、光学异构体等等),如可由于各种取代基上的不对称碳而存在的那些立体异构体,包括对映异构形式(其可甚至在不存在不对称碳的情况下存在)、旋转异构形式、阻转异构体和非对映异构形式,涵盖在本公开内容的范围内。本公开内容的化合物的个别立体异构体可例如基本上不含其它异构体,或可例如以外消旋体形式或与所有其它或其它所选立体异构体混合。本文中化合物的手性中心可具有如由IUPAC 1974建议所定义的S或R构型。术语"盐"、"溶剂合物"等等的使用预期同样适用于所公开的化合物的对映异构体、立体异构体、旋转异构体、互变异构体、外消旋体或前药的盐和溶剂合物。

[0142] 可用作化学治疗剂(抗肿瘤剂)的化合物的类别包括:烷基化剂、抗代谢物、天然产品和其衍生物、激素和类固醇(包括合成类似物)以及合成物。下文给出这些类别内的化合物的实例。

[0143] 脂质粒子

[0144] 本说明书提供包含囊封在脂质粒子内的一种或多种治疗性mRNA分子的脂质粒子。

[0145] 在一些实施例中,mRNA完全囊封在脂质粒子的脂质部分内,以使得脂质粒子中的mRNA在水溶液中抗核酸酶降解。在其它实施例中,本文所描述的脂质粒子对如人类等哺乳动物基本上无毒性。脂质粒子的平均直径通常为30nm至150nm、40nm至150nm、50nm至150nm、60nm至130nm、70nm至110nm或70至90nm。本发明的脂质粒子的脂质:RNA比率(质量/质量比)还通常为1:1至100:1、1:1至50:1、2:1至25:1、3:1至20:1、5:1至15:1、或5:1至10:1、或10:1至14:1、或9:1至20:1。在一个实施例中,脂质粒子的脂质:RNA比率(质量/质量比)为12:1。在另一个实施例中,脂质粒子的脂质:mRNA比率(质量/质量比)为13:1。

[0146] 在优选实施例中,脂质粒子包含mRNA、阳离子脂质(例如,本文所描述的一种或多种阳离子脂质或其盐)、磷脂和抑制粒子聚集的结合脂质(例如,一种或多种PEG-脂质结合物)。脂质粒子还可包括胆固醇。脂质粒子可包含至少1、2、3、4、5、6、7、8、9、10个或更多表达一种或多种多肽的mRNA。

[0147] 在核酸-脂质粒子中,mRNA可完全囊封在粒子的脂质部分内,从而保护核酸不被核酸酶降解。在优选实施例中,包含mRNA的脂质粒子完全囊封在粒子的脂质部分内,从而保护核酸不被核酸酶降解。在某些情况下,粒子在37℃下暴露于核酸酶至少20、30、45或60分钟之后,脂质粒子中的mRNA基本上并未降解。在某些其它情况下,粒子在血清中在37℃下培育至少30、45或60分钟或至少2、3、4、5、6、7、8、9、10、12、14、16、18、20、22、24、26、28、30、32、34或36小时之后,脂质粒子中的mRNA基本上并未降解。在其它实施例中,mRNA与粒子的脂质部分复合。本发明配制品的一个益处是核酸-脂质粒子组合物对如人类等哺乳动物基本上无毒性。

[0148] "完全囊封"意味着核酸-脂质粒子中的核酸(例如,mRNA)在暴露于将显著降解游离RNA的血清或核酸酶分析法之后并未显著降解。当完全囊封时,粒子中优选地小于25%的核酸在通常将降解100%游离核酸的处理中被降解,粒子中更优选地小于10%并且最优选地小于5%的核酸被降解。"完全囊封"还意味着核酸-脂质粒子在活体内投与后不会快速分解成其组成部分。

[0149] 在核酸的情形下,完全囊封可通过进行不可透过膜的荧光染料排阻分析法来测

定，所述分析法使用当与核酸缔合时荧光增强的染料。囊封是通过添加染料至脂质体配制品，测量所得荧光并且比较其与在添加少量非离子型清洁剂后所观测到的萤光来测定。清洁剂介导的脂质体双层的破裂释放所囊封的核酸，从而使其与不可透过膜的染料相互作用。核酸囊封可如下计算：$E=(I_0-I)/I_0$，其中I和I_0是指清洁剂添加前后的荧光强度。

[0150] 在其它实施例中，本发明提供包含多个核酸-脂质粒子的核酸-脂质粒子组合物。

[0151] 脂质粒子包含完全囊封在粒子的脂质部分内的mRNA，以使得30％至100％、40％至100％、50％至100％、60％至100％、70％至100％、80％至100％、90％至100％、30％至95％、40％至95％、50％至95％、60％至95％、70％至95％、80％至95％、85％至95％、90％至95％、30％至90％、40％至90％、50％至90％、60％至90％、70％至90％、80％至90％、或至少30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％(或其任何分数或其中的范围)的粒子其中囊封有mRNA。

[0152] 视脂质粒子的既定用途而定，可改变组分的比例并且可使用所属领域中已知分析法测量特定配制品的递送效率。

[0153] 阳离子脂质

[0154] 本说明书包括某些阳离子脂质化合物的合成。所述化合物特别适用于递送聚核苷酸至细胞和组织，如后续部分所展现。本文所描述的脂质大环化合物可用于其它目的以及例如受体和添加剂。

[0155] 阳离子脂质化合物的合成方法可使用所属领域中的技能合成。所属领域的技术人员将发现其它方法以制造这些化合物以及制造本说明书的其它化合物。

[0156] 阳离子脂质化合物可与药剂组合以形成微米粒子、纳米粒子、脂质体或胶束。有待通过粒子、脂质体或胶束递送的药剂可呈气体、液体或固体形式。并且药剂可为聚核苷酸、蛋白质、肽或小分子。脂质大环化合物可与其它阳离子脂质化合物、聚合物(合成或天然)、表面活性剂、胆固醇、碳水化合物、蛋白质或脂质组合以形成粒子。这些粒子可随后任选地与医药赋形剂组合以形成医药组合物。

[0157] 本说明书提供新颖的阳离子脂质化合物和基于这类阳离子脂质化合物的使用的药物递送系统。所述系统可用于医药/药物递送技术中以递送聚核苷酸、蛋白质、小分子、肽、抗原或药物至患者、组织、器官或细胞。这些新颖化合物还可用作用于涂布、添加剂、赋形剂、材料或生物工程的材料。

[0158] 本说明书的阳离子脂质化合物提供药物递送技术中的数种不同用途。阳离子脂质化合物的含胺部分可用于与聚核苷酸复合，从而增强聚核苷酸的递送并且防止其降解。阳离子脂质化合物还可用于形成含有有待递送的药剂的皮粒子、纳米粒子、微米粒子、脂质体和胶束。优选地，阳离子脂质化合物是生物相容和可生物降解的，并且所形成的粒子也是可生物降解和生物相容的并且可用于受控的持续释放有待递送的药剂。鉴于这些阳离子脂质化合物在较低pH下质子化，这些阳离子脂质化合物和其相应粒子也响应于pH变化。它们也可在药剂递送至细胞时充当质子海绵以引起内体溶解。

[0159] 在某些实施例中，阳离子脂质化合物是相对无细胞毒性的。阳离子脂质化合物可为生物相容和可生物降解的。阳离子脂质的pK_a可在大致5.5至大致7.5范围内，更优选地在大致6.0与大致7.0之间。其可经设计以使得所期望的pK_a在大致3.0与大致9.0之间，或在大致5.0与大致8.0之间。本文所描述的阳离子脂质化合物出于以下若干原因而对药物递送特

别具有吸引力:其含有氨基以便与DNA、RNA其它聚核苷酸和其它带负电荷药剂相互作用、缓冲pH、引起内渗透、保护有待递送的药剂,其可由可商购的起始物质合成;和/或其具有pH响应性并且可经工程改造以具有所期望的pK_a。

[0160] 含有阳离子脂质化合物的组合物可为30-70%阳离子脂质化合物、0-60%胆固醇、0-30%磷脂和1-10%聚乙二醇(PEG)。优选地,所述组合物是30-40%阳离子脂质化合物、40-50%胆固醇和10-20%PEG。在其它优选实施例中,所述组合物是50-75%阳离子脂质化合物、20-40%胆固醇和5-10%磷脂和1-10%PEG。所述组合物可含有60-70%阳离子脂质化合物、25-35%胆固醇和5-10%PEG。所述组合物可含有多达90%阳离子脂质化合物和2-15%辅助脂质。

[0161] 配制品可为脂质粒子配制品,例如含有8-30%化合物、5-30%辅助脂质和0-20%胆固醇;4-25%阳离子脂质、4-25%辅助脂质、2-25%胆固醇、10-35%胆固醇-PEG和5%胆固醇-胺;或2-30%阳离子脂质、2-30%辅助脂质、1-15%胆固醇、2-35%胆固醇-PEG和1-20%胆固醇-胺;或多达90%阳离子脂质和2-10%辅助脂质;或甚至100%阳离子脂质。

[0162] 非阳离子脂质

[0163] 脂质粒子中所用的非阳离子脂质可为各种能够制造稳定复合物的中性不带电脂质、两性离子脂质或阴离子脂质中的任一种。

[0164] 非阳离子脂质的非限制性实例包括磷脂,如卵磷脂、磷脂酰乙醇胺、溶血卵磷脂、溶血磷脂酰乙醇胺、磷脂酰丝氨酸、磷脂酰肌醇、鞘磷脂、卵鞘磷脂(ESM)、脑磷脂、心磷脂、磷脂酸、脑苷脂、二鲸蜡基磷酸酯、二硬脂酰基磷脂酰胆碱(DSPC)、二油酰基磷脂酰胆碱(DOPC)、二棕榈酰基磷脂酰胆碱(DPPC)、二油酰基磷脂酰甘油(DOPG)、二棕榈酰基磷脂酰甘油(DPPG)、二油酰基磷脂酰乙醇胺(DOPE)、棕榈酰油酰基-磷脂酰胆碱(POPC)、棕榈酰油酰基-磷脂酰乙醇胺(POPE)、棕榈酰油酰基-磷脂酰甘油(POPG)、二油酰基磷脂酰乙醇胺4-(N-马来酰亚胺基甲基)-环己烷-1-甲酸酯(DOPE-mal)、二棕榈酰基-磷脂酰乙醇胺(DPPE)、二肉豆蔻酰基-磷脂酰乙醇胺(DMPE)、二硬脂酰基-磷脂酰乙醇胺(DSPE)、单甲基-磷脂酰乙醇胺、二甲基-磷脂酰乙醇胺、二反式油酰基-磷脂酰乙醇胺(DEPE)、硬脂酰油酰基-磷脂酰乙醇胺(SOPE)、溶血磷脂酰胆碱、二亚油酰磷脂酰胆碱和其混合物。还可使用其它二酰基磷脂酰胆碱和二酰基磷脂酰乙醇胺磷脂。这些脂质中的酰基优选地是来源于具有C_{10}-C_{24}碳链的脂肪酸的酰基,例如月桂酰基、肉豆蔻酰基、棕榈酰基、硬脂酰基或油酰基。

[0165] 非阳离子脂质的额外实例包括固醇,如胆固醇和其衍生物。胆固醇衍生物的非限制性实例包括极性类似物,如5α-胆甾烷醇、5α-粪甾醇、胆固醇基-(2′-羟基)-乙基醚、胆固醇基-(4′-羟基)-丁基醚和6-酮胆甾烷醇;非极性类似物,如5α-胆甾烷、胆甾烯酮、5α-胆甾烷酮、5α-胆甾烷酮和胆固醇基癸酸酯;和其混合物。在优选实施例中,胆固醇衍生物是极性类似物,如胆固醇基-(4′-羟基)-丁基醚。

[0166] 在一些实施例中,脂质粒子中所存在的非阳离子脂质包含一种或多种磷脂和胆固醇或其衍生物的混合物或由一种或多种磷脂和胆固醇或其衍生物的混合物组成。在其它实施例中,脂质粒子中所存在的非阳离子脂质包含一种或多种磷脂或由一种或多种磷脂组成,例如无胆固醇的脂质粒子配制品。在又其它实施例中,脂质粒子中所存在的非阳离子脂质包含胆固醇或其衍生物或由胆固醇或其衍生物组成,例如无磷脂的脂质粒子配制品。

[0167] 非阳离子脂质的其它实例包括不含磷的脂质,如硬脂胺、十二烷胺、十六烷胺、乙

酰基棕榈酸酯、甘油蓖麻醇酸酯、硬脂酸十六烷酯、肉豆蔻酸异丙酯、两性丙烯酸聚合物、月桂基硫酸三乙醇胺、烷基-芳基硫酸聚乙氧基化脂肪酸酰胺、双十八烷基二甲基溴化铵、神经酰胺和鞘磷脂。

[0168] 在一些实施例中，非阳离子脂质占粒子中所存在的总脂质的10摩尔％至60摩尔％、20摩尔％至55摩尔％、20摩尔％至45摩尔％、20摩尔％至40摩尔％、25摩尔％至50摩尔％、25摩尔％至45摩尔％、30摩尔％至50摩尔％、30摩尔％至45摩尔％、30摩尔％至40摩尔％、35摩尔％至45摩尔％、37摩尔％至42摩尔％、或35摩尔％、36摩尔％、37摩尔％、38摩尔％、39摩尔％、40摩尔％、41摩尔％、42摩尔％、43摩尔％、44摩尔％或45摩尔％(或其任何分数或其中的范围)。

[0169] 在脂质粒子含有磷脂和胆固醇或胆固醇衍生物的混合物的实施例中，混合物可占粒子中所存在的总脂质的多达40摩尔％、45摩尔％、50摩尔％、55摩尔％或60摩尔％。

[0170] 在一些实施例中，混合物中的磷脂组分可占粒子中所存在的总脂质的2摩尔％至20摩尔％、2摩尔％至15摩尔％、2摩尔％至12摩尔％、4摩尔％至15摩尔％或4摩尔％至10摩尔％(或其任何分数或其中的范围)。在某些优选实施例中，混合物中的磷脂组分占粒子中所存在的总脂质的5摩尔％至10摩尔％、5摩尔％至9摩尔％、5摩尔％至8摩尔％、6摩尔％至9摩尔％、6摩尔％至8摩尔％、或5摩尔％、6摩尔％、7摩尔％、8摩尔％、9摩尔％或10摩尔％(或其任何分数或其中的范围)。

[0171] 在其它实施例中，混合物中的胆固醇组分可占粒子中所存在的总脂质的25摩尔％至45摩尔％、25摩尔％至40摩尔％、30摩尔％至45摩尔％、30摩尔％至40摩尔％、27摩尔％至37摩尔％、25摩尔％至30摩尔％或35摩尔％至40摩尔％(或其任何分数或其中的范围)。在某些优选实施例中，混合物中的胆固醇组分占粒子中所存在的总脂质的25摩尔％至35摩尔％、27摩尔％至35摩尔％、29摩尔％至35摩尔％、30摩尔％至35摩尔％、30摩尔％至34摩尔％、31摩尔％至33摩尔％、或30摩尔％、31摩尔％、32摩尔％、33摩尔％、34摩尔％或35摩尔％(或其任何分数或其中的范围)。

[0172] 在脂质粒子无磷脂的实施例中，胆固醇或其衍生物可占粒子中所存在的总脂质的多达25摩尔％、30摩尔％、35摩尔％、40摩尔％、45摩尔％、50摩尔％、55摩尔％或60摩尔％。

[0173] 在一些实施例中，无磷脂的脂质粒子配制品中的胆固醇或其衍生物可占粒子中所存在的总脂质的25摩尔％至45摩尔％、25摩尔％至40摩尔％、30摩尔％至45摩尔％、30摩尔％至40摩尔％、31摩尔％至39摩尔％、32摩尔％至38摩尔％、33摩尔％至37摩尔％、35摩尔％至45摩尔％、30摩尔％至35摩尔％、35摩尔％至40摩尔％、或30摩尔％、31摩尔％、32摩尔％、33摩尔％、34摩尔％、35摩尔％、36摩尔％、37摩尔％、38摩尔％、39摩尔％或40摩尔％(或其任何分数或其中的范围)。

[0174] 在其它实施例中，非阳离子脂质占粒子中所存在的总脂质的5摩尔％至90摩尔％、10摩尔％至85摩尔％、20摩尔％至80摩尔％、10摩尔％(例如，仅磷脂)或60摩尔％(例如，磷脂和胆固醇或其衍生物)(或其任何分数或其中的范围)。

[0175] 脂质粒子中所存在的非阳离子脂质的百分比是目标量，并且配制品中所存在的非阳离子脂质的实际量可变化例如±5摩尔％。

[0176] 脂质结合物

[0177] 除阳离子以外，本文所描述的脂质粒子可另外包含脂质结合物。结合脂质是有用

的,因为其防止粒子聚集。合适的结合脂质包括(但不限于)PEG-脂质结合物、阳离子-聚合物-脂质结合物和其混合物。

[0178] 在一优选实施例中,脂质结合物是PEG-脂质。PEG-脂质的实例包括(但不限于)偶合到二烷氧基丙基的PEG(PEG-DAA)、偶合到二酰基甘油的PEG(PEG-DAG)、偶合到如磷脂酰乙醇胺等磷脂的PEG(PEG-PE)、结合到神经酰胺的PEG、结合到胆固醇或其衍生物的PEG和其混合物。

[0179] PEG是具有两个末端羟基的亚乙基PEG重复单元的水溶性线性聚合物。PEG是通过其分子量分类;并且包括以下:单甲氧基聚乙二醇(MePEG-OH)、单甲氧基聚乙二醇-丁二酸酯(MePEG-S)、单甲氧基聚乙二醇-丁二酰亚胺基丁二酸酯(MePEG-S-NHS)、单甲氧基聚乙二醇-胺(MePEG-NH_2)、单甲氧基聚乙二醇-三氟乙磺酸酯(MePEG-TRES)、单甲氧基聚乙二醇-咪唑基-羰基(MePEG-IM)以及含有末端羟基而非末端甲氧基的这类化合物(例如,HO-PEG-S、HO-PEG-S-NHS、HO-PEG-NH_2)。

[0180] 本文所描述的PEG-脂质结合物的PEG部分可包含范围介于550道尔顿至10,000道尔顿的平均分子量。在某些情况下,PEG部分的平均分子量为750道尔顿至5,000道尔顿(例如,1,000道尔顿至5,000道尔顿、1,500道尔顿至3,000、750道尔顿至3,000道尔顿、750道尔顿至2,000道尔顿)。在优选实施例中,PEG部分的平均分子量为2,000道尔顿或750道尔顿。

[0181] 在某些情况下,PEG可任选地经烷基、烷氧基、酰基或芳基取代。PEG可直接结合到脂质或可经由连接体部分连接到脂质。可使用适用于将PEG偶合到脂质的任何连接体部分,包括例如不含酯的连接体部分和含有酯的连接体部分。在一优选实施例中,连接体部分是不含酯的连接体部分。合适的不含酯的连接体部分包括(但不限于)酰胺基(-C(O)NH-)、氨基(-NR-)、羰基(-C(O)-)、氨基甲酸酯基(-NHC(O)O-)、脲(-NHC(O)NH-)、二硫化物(-S-S-)、醚(-O-)、丁二酰基(-(O)CCH_2CH_2C(O)-)、丁二酰胺基(-NHC(O)CH_2CH_2C(O)NH-)、醚、二硫化物以及其组合(如含有氨基甲酸酯基连接体部分和酰胺基连接体部分的连接体)。在一优选实施例中,使用氨基甲酸酯基连接体将PEG偶合到脂质。

[0182] 在其它实施例中,使用含有酯的连接体部分将PEG偶合到脂质。合适的含有酯的连接体部分包括例如碳酸酯基(-OC(O)O-)、丁二酰基、磷酸酯(-O-(O)POH-O-)、磺酸酯和其组合。

[0183] 具有各种不同链长度和饱和度的酰基链基团的磷脂酰乙醇胺可结合到PEG以形成脂质结合物。这类磷脂酰乙醇胺是可商购的,或可使用所属领域的技术人员已知的常规技术分离或合成。含有碳链长度在C_{10}至C_{20}范围内的饱和或不饱和脂肪酸的磷脂酰乙醇胺是优选的。还可使用具有单或二不饱和脂肪酸和饱和与不饱和脂肪酸的混合物的磷脂酰乙醇胺。合适的磷脂酰乙醇胺包括(但不限于)二肉豆蔻酰基-磷脂酰乙醇胺(DMPE)、二棕榈酰基-磷脂酰乙醇胺(DPPE)、二油酰基-磷脂酰乙醇胺(DOPE)和二硬脂酰基-磷脂酰乙醇胺(DSPE)。

[0184] 术语“二酰基甘油”或“DAG”包括具有2个脂肪酰基链R^1和R^2的化合物,R^1和R^2都独立地具有通过酯键键结于甘油的1-和2-位置的2至30个碳。酰基可为饱和的或具有不同的不饱和度。合适的酰基包括(但不限于)月桂酰基(C_{12})、肉豆蔻酰基(C_{14})、棕榈酰基(C_{16})、硬脂酰基(C_{18})和花生酰基(C_{20})。在优选实施例中,R^1和R^2是相同的,即R^1和R^2都是肉豆蔻酰基(即,二肉豆蔻酰基),R^1和R^2都是硬脂酰基(即,二硬脂酰基)。

[0185] 术语“二烷氧基丙基”或“DAA”包括具有2个烷基链R和R的化合物，R和R都独立地具有2至30个碳。烷基可为饱和的或具有不同的不饱和度。

[0186] 优选地，PEG-DAA结合物是PEG-二癸氧基丙基(C_{10})结合物、PEG-二月桂基氧基丙基(C_{12})结合物、PEG-二肉豆蔻氧基丙基(C_{14})结合物、PEG-二棕榈氧基丙基(C_{16})结合物或PEG-二硬脂氧基丙基(C_{18})结合物。在这些实施例中，PEG的平均分子量优选地是750或2,000道尔顿。在特定实施例中，PEG的末端羟基经甲基取代。

[0187] 除上述以外，可使用其它亲水性聚合物代替PEG。可用于代替PEG的合适聚合物的实例包括(但不限于)聚乙烯吡咯烷酮、聚甲基噁唑啉、聚乙基噁唑啉、聚羟丙基甲基丙烯酰胺、聚甲基丙烯酰胺和聚二甲基丙烯酰胺、聚乳酸、聚乙醇酸和衍生纤维素，如羟甲基纤维素或羟乙基纤维素。

[0188] 在一些实施例中，脂质结合物(例如，PEG-脂质)占粒子中所存在的总脂质的0.1摩尔％至2摩尔％、0.5摩尔％至2摩尔％、1摩尔％至2摩尔％、0.6摩尔％至1.9摩尔％、0.7摩尔％至1.8摩尔％、0.8摩尔％至1.7摩尔％、0.9摩尔％至1.6摩尔％、0.9摩尔％至1.8摩尔％、1摩尔％至1.8摩尔％、1摩尔％至1.7摩尔％、1.2摩尔％至1.8摩尔％、1.2摩尔％至1.7摩尔％、1.3摩尔％至1.6摩尔％或1.4摩尔％至1.5摩尔％(或其任何分数或其中的范围)。在其它实施例中，脂质结合物(例如，PEG-脂质)占粒子中所存在的总脂质的0摩尔％至20摩尔％、0.5摩尔％至20摩尔％、2摩尔％至20摩尔％、1.5摩尔％至18摩尔％、2摩尔％至15摩尔％、4摩尔％至15摩尔％、2摩尔％至12摩尔％、5摩尔％至12摩尔％或2摩尔％(或其任何分数或其中的范围)。

[0189] 在其它实施例中，脂质结合物(例如，PEG-脂质)占粒子中所存在的总脂质的4摩尔％至10摩尔％、5摩尔％至10摩尔％、5摩尔％至9摩尔％、5摩尔％至8摩尔％、6摩尔％至9摩尔％、6摩尔％至8摩尔％、或5摩尔％、6摩尔％、7摩尔％、8摩尔％、9摩尔％或10摩尔％(或其任何分数或其中的范围)。

[0190] 本发明的脂质粒子中所存在的脂质结合物(例如，PEG-脂质)的百分比是目标量，并且配制品中所存在的脂质结合物的实际量可变化例如±2摩尔％。所属领域的一般技术人员应了解，脂质结合物的浓度可根据所采用的脂质结合物和脂质粒子变得促融的速率而变化。

[0191] 通过控制脂质结合物的组成和浓度，有人可控制脂质结合物从脂质粒子交换出的速率并且，继而可控制脂质粒子变得促融的速率。另外，可使用其它变量改变和/或控制脂质粒子变得促融的速率，包括例如pH、温度或离子强度。在阅读本公开内容后，可用于控制脂质粒子变得促融的速率的其它方法将变得对所属领域的技术人员显而易见。另外，通过控制脂质结合物的组成和浓度，有人可控制脂质粒度。

[0192] 用于投与的组合物和配制品

[0193] 本公开内容的核酸-脂质组合物可通过多种途径投与，例如经由静脉内、肠胃外、腹膜内或局部途径实现全身递送。在一些实施例中，可在细胞内，例如在如肺或肝脏等目标组织的细胞中或在发炎组织中递送siRNA。在一些实施例中，本公开内容提供一种用于活体内递送siRNA的方法。可将核酸-脂质组合物静脉内、皮下或腹膜内投与个体。在一些实施例中，本公开内容提供活体内递送干扰RNA至哺乳动物个体的肺的方法。

[0194] 在一些实施例中，本公开内容提供一种治疗哺乳动物个体的疾病或病症的方法。

治疗有效量的本公开内容的含有核、阳离子脂质、两亲分子、磷脂、胆固醇和PEG连接的胆固醇的组合物可投与患有与可通过所述组合物减少、降低、下调或沉默的基因的表达或过度表达相关联的疾病或病症的个体。

[0195] 可通过多种粘膜投与模式，包括通过经口、经直肠、经阴道、鼻内、肺内或透皮或经皮递送，或通过局部递送至眼、耳、皮肤或其它粘膜表面而向个体投与本公开内容的组合物和方法。在本公开内容的一些方面中，粘膜组织层包括上皮细胞层。上皮细胞可为肺部、气管、支气管、肺泡、鼻、口腔、表皮或胃肠道上皮细胞。本公开内容的组合物可使用常规致动器(如机械喷雾装置)以及加压、电激活或其它类型的致动器投与。

[0196] 本公开内容的组合物可在水溶液中以经鼻或肺部喷雾形式投与，并且可通过所属领域的技术人员已知的各种方法以喷雾形式分配。本公开内容的组合物的肺部递送是通过投与可例如经气溶胶化、雾化或喷雾而呈滴剂、粒子或喷雾形式的组合物来实现。组合物的粒子、喷雾或气溶胶可呈液体或固体形式。优选的用于以经鼻喷雾形式分配液体的系统公开在美国专利第4,511,069号中。这类配制品可便利地通过将根据本公开内容的组合物溶解于水中以制造水溶液并且使得所述溶液无菌而制备。配制品可存在于多剂量容器中，例如在美国专利第4,511,069号中所公开的密封分配系统中。其它合适的经鼻喷雾递送系统已描述于透皮全身性药物(Transdermal Systemic Medication)，Y.W.Chien编，纽约爱思唯尔出版社(Elsevier Publishers,New York)，1985;和美国专利第4,778,810号中。额外气溶胶递送形式可包括例如压缩空气、喷射、超声和压电喷雾器，其递送溶解或悬浮于医药溶剂(例如水、乙醇或其混合物)中的生物活性剂。

[0197] 本公开内容的经鼻和经肺喷雾溶液通常包含药物或有待递送的药物，其任选地用表面活性剂，如非离子型表面活性剂(例如聚山梨醇酯-80)和一种或多种缓冲液配制。在本公开内容的一些实施例中，经鼻喷雾溶液另外包含推进剂。经鼻喷雾溶液的pH可为pH 6.8至7.2。所采用的医药溶剂也可为pH 4-6的微酸性水性缓冲液。可添加其它组分以增强或维持化学稳定性，包括防腐剂、表面活性剂、分散剂或气体。

[0198] 在一些实施例中，本公开内容是一种医药产品，其包括含有本公开内容的组合物的溶液和用于肺部、粘膜或鼻内喷雾或气溶胶的致动器。

[0199] 本公开内容的组合物的剂型可为液体，呈滴剂或乳液形式或呈气溶胶形式。

[0200] 本公开内容的组合物的剂型可为固体，其可在投与之前在液体中复原。固体可以粉末形式投与。固体可呈胶囊、片剂或凝胶形式。

[0201] 为了配制在本公开内容内用于经肺递送的组合物，可将生物活性剂与多种药学上可接受的添加剂以及用于分散活性剂的基质或载剂组合。添加剂的实例包括pH控制剂，如精氨酸、氢氧化钠、甘氨酸、盐酸、柠檬酸和其混合物。其它添加剂包括局部麻醉剂(例如，苯甲醇)、等张剂(例如，氯化钠、甘露醇、山梨醇)、吸附抑制剂(例如吐温80)、溶解度增强剂(例如，环糊精和其衍生物)、稳定剂(例如，血清白蛋白)和还原剂(例如，谷胱甘肽)。当用于粘膜递送的组合物是液体时，如参照视为单位的0.9％(w/v)生理盐水溶液的张力所测量，配制品的张力通常调节至在投与位点处将基本上不诱发粘膜的不可逆组织损伤的值。一般来说，溶液的张力调节至1/3至3、更通常1/2至2并且最通常3/4至1.7的值。

[0202] 生物活性剂可分散于基质或媒剂中，所述基质或媒剂可包含具有分散活性剂和任何所需添加剂的能力的亲水性化合物。基质可选自广泛范围的合适载剂，其包括(但不限

于)聚羧酸或其盐、羧酸酐(例如,顺丁烯二酸酐)与其它单体(例如,(甲基)丙烯酸甲酯、丙烯酸等)的共聚物;亲水性乙烯聚合物,如聚乙酸乙烯酯、聚乙烯醇、聚乙烯吡咯烷酮;纤维素衍生物,如羟甲基纤维素、羟丙基纤维素等;和天然聚合物,如壳聚糖、胶原蛋白、海藻酸钠、明胶、透明质酸和其无毒性金属盐。通常,选择可生物降解的聚合物作为基质或载剂,例如聚乳酸、聚(乳酸-乙醇酸)共聚物、聚羟基丁酸、聚(羟基丁酸-乙醇酸)共聚物和其混合物。或者或另外,可使用合成脂肪酸酯作为载剂,如聚甘油脂肪酸酯、蔗糖脂肪酸酯等。亲水性聚合物和其它载剂可单独或组合使用,并且可通过部分结晶、离子键结、交联等等赋予载剂增强的结构完整性。载剂可以各种形式提供,包括流体或粘稠溶液、凝胶、糊剂、粉末、微球体和膜,以便直接施用于鼻粘膜。在此情形中使用所选载剂可促进生物活性剂的吸收。

[0203] 用于粘膜、经鼻或经肺递送的配制品可含有亲水性低分子量化合物作为基质或赋形剂。这类亲水性低分子量化合物提供通过介质,经由其如生理学活性肽或蛋白质等水溶性活性剂可扩散穿过基质到活性剂所吸附的体表。亲水性低分子量化合物任选地从粘膜或投与气氛吸收水分并且溶解水溶性活性肽。亲水性低分子量化合物的分子量一般不超过10,000并且优选地不超过3,000。亲水性低分子量化合物的实例包括多元醇化合物,如寡糖、二糖和单糖,包括蔗糖、甘露醇、乳糖、L-阿拉伯糖、D-赤藓糖、D-核糖、D-木糖、D-甘露糖、D-半乳糖、乳果糖、纤维二糖、龙胆二糖、甘油、聚乙二醇和其混合物。亲水性低分子量化合物的其它实例包括N-甲基吡咯烷酮、醇(例如,寡聚乙烯醇、乙醇、乙二醇、丙二醇等)和其混合物。

[0204] 本公开内容的组合物或者可视需要含有药学上可接受的载剂物质以近似生理条件,如pH调节和缓冲剂、张力调节剂和湿润剂,例如乙酸钠、乳酸钠、氯化钠、氯化钾、氯化钙、脱水山梨糖醇单月桂酸酯、三乙醇胺油酸酯和其混合物。对于固体组合物,可使用常规无毒的药学上可接受的载剂,其包括例如药物级甘露醇、乳糖、淀粉、硬脂酸镁、糖精钠、滑石、纤维素、葡萄糖、蔗糖、碳酸镁等等。

[0205] 在本公开内容的某些实施例中,生物活性剂可在延时释放配制品中,例如在包括缓慢释放聚合物的组合物中投与。活性剂可与将避免快速释放的载剂一起制备,例如控制释放媒剂,如聚合物、微囊封递送系统或生物粘附性凝胶。本公开内容的各种组合物中活性剂的延长递送可通过在组合物中包括延迟吸收剂(例如,单硬脂酸铝水凝胶和明胶)而实现。

[0206] 虽然已关于某些实施例描述本公开内容并且已出于说明的目的阐述许多详情,但是本公开内容包括额外实施例并且本文所描述的一些详情可在不脱离本公开内容的情况下显著改变将对所属领域的技术人员显而易见。本公开内容包括这类额外实施例、修改和等效物。具体来说,本公开内容包括各种说明性组分和实例的特征、术语或要素的任何组合。

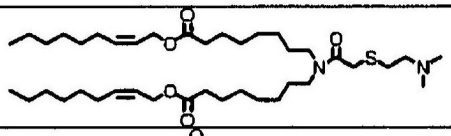
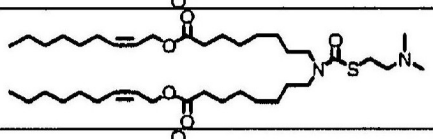
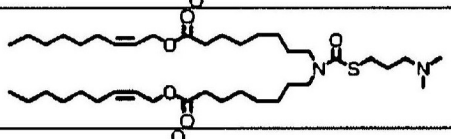
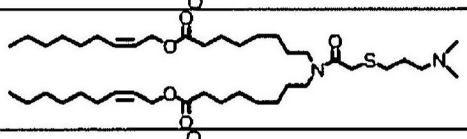
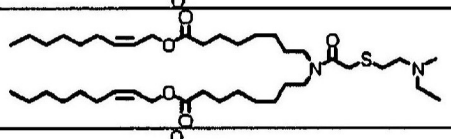
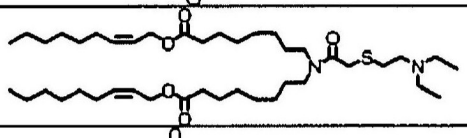
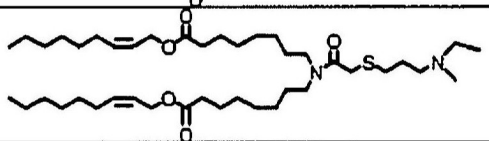
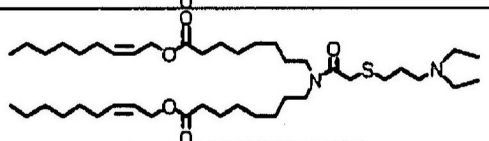
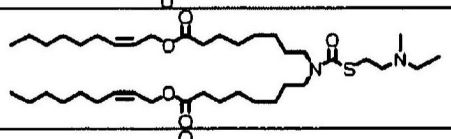
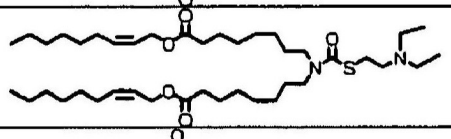
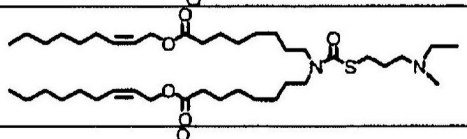
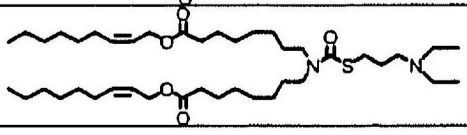
[0207] 实例

[0208] 实例1.

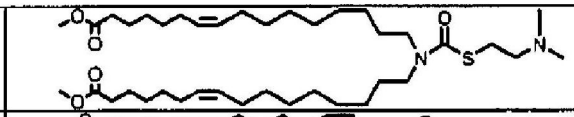
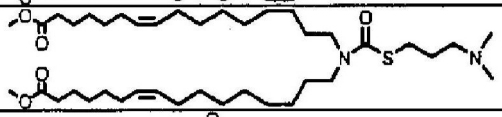
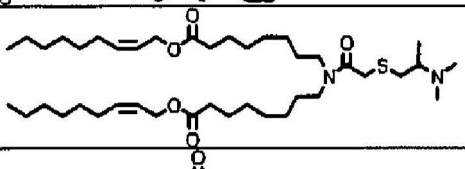
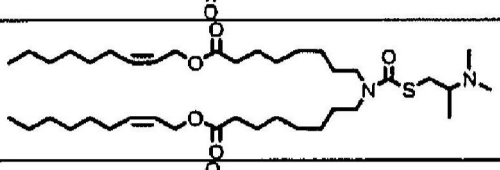
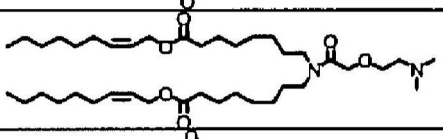
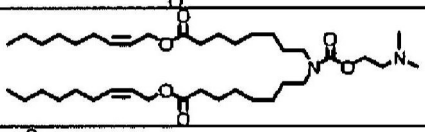
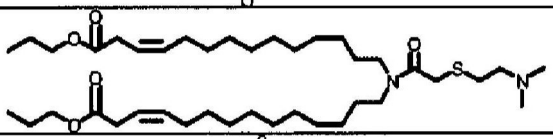
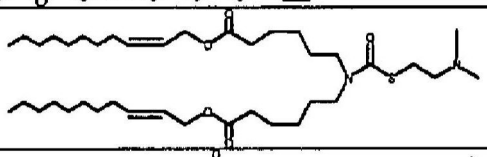
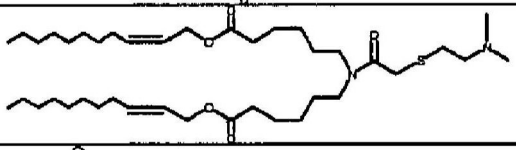
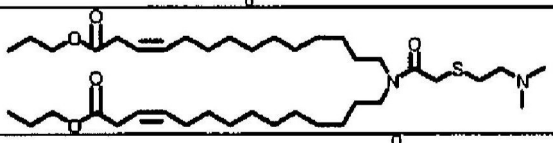
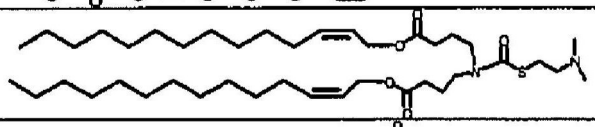
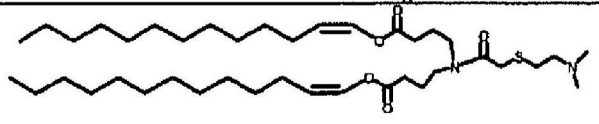
[0209] 示例性式I化合物提供于表1中。

[0210] 表1

[0211]

脂质编号	新颖脂质	MW	pKa	在0.3 mg/kg下的KD
ATX-001		695.1	8.9	~0
ATX-002		681	8.7	98
ATX-003		695.1	9.3	~0
ATX-004		709.13	9.4	~0
ATX-005		709.13	9.0	~0
ATX-006		723.15	9.8	~0
ATX-007		723.15	9.5	n/a
ATX-008		737.18	10.3	n/a
ATX-009		695.1	8.8	~0
ATX-010		709.13	9.6	30
ATX-011		709.13	9.4	n/a
ATX-012		723.15	10.2	~0

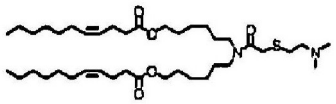
[0212]

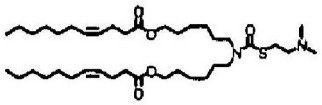
ATX-013		681.01		n/a
ATX-014		695.1		n/a
ATX-015		695.1		n/a
ATX-016		709.13		15
ATX-017		695.1		n/a
ATX-021		679.04		n/a
ATX-022		665.01		n/a
ATX-023		695.1		n/a
ATX-026		681.07		n/a
ATX-027		695.1		n/a
ATX-028		681.07		n/a
ATX-029		681.1		n/a
ATX-030		695.1		n/a

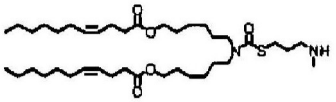
[0213] 表1示出了每种化合物的名称和结构、其分子量、其pKa和其在下文实例19中所述的分析法中的敲低生物活性(KD)。

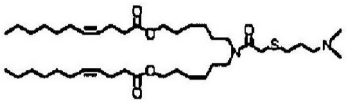
[0214] 示例性式II和III化合物提供于表2和3中。

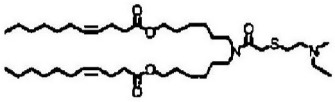
[0215] 表2

1) 

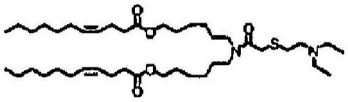
2) 

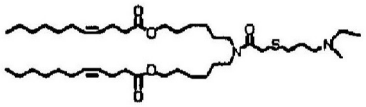
3) 

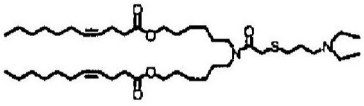
4) 

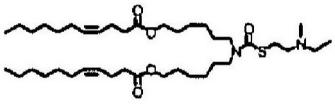
5) 

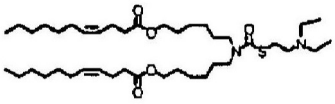
[0216]

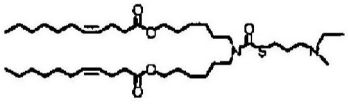
6) 

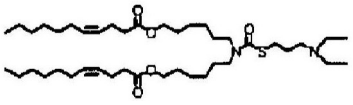
7) 

8) 

9) 

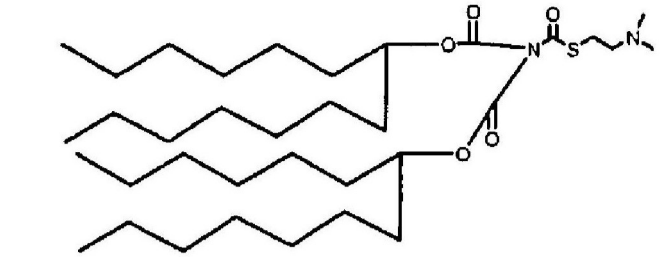
10) 

11) 

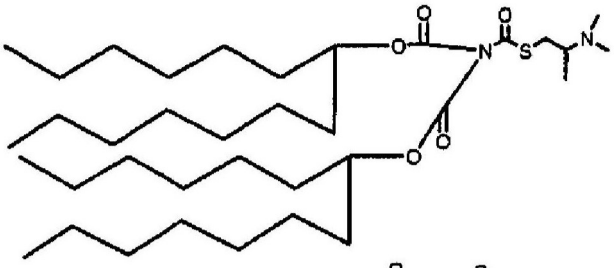
12) 

[0217] 表3

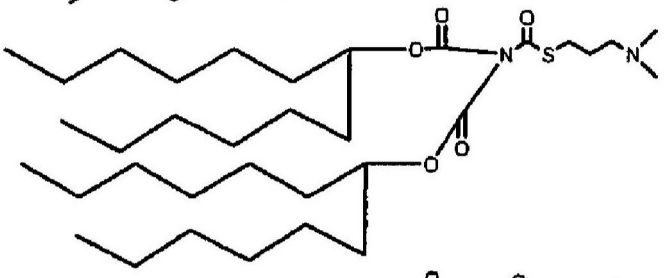
[0218]



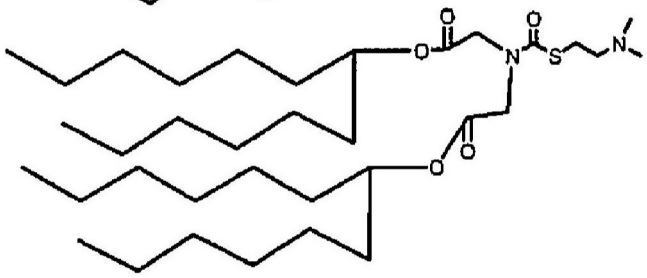
ATX-B-1



ATX-B-2

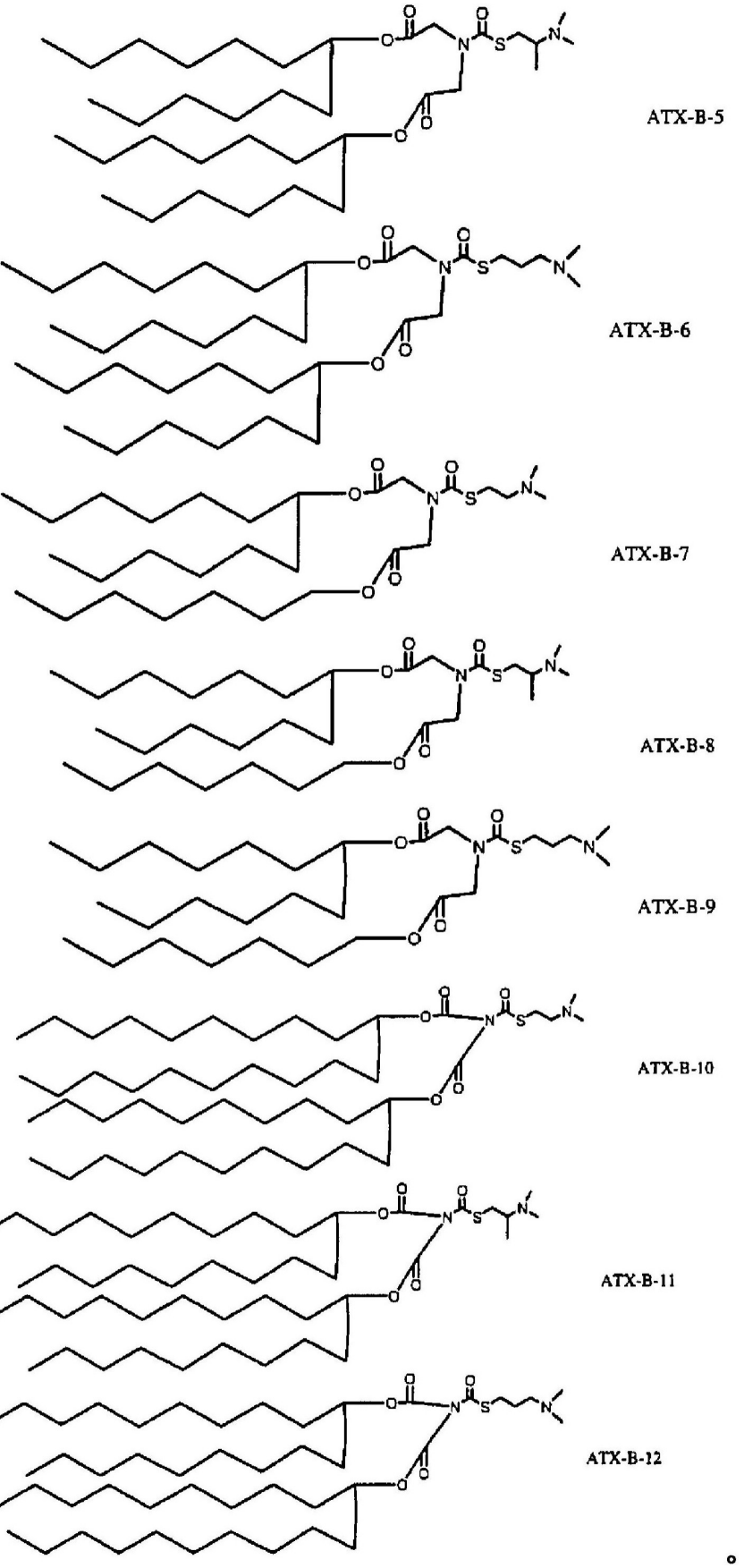


ATX-B-3



ATX-B-4

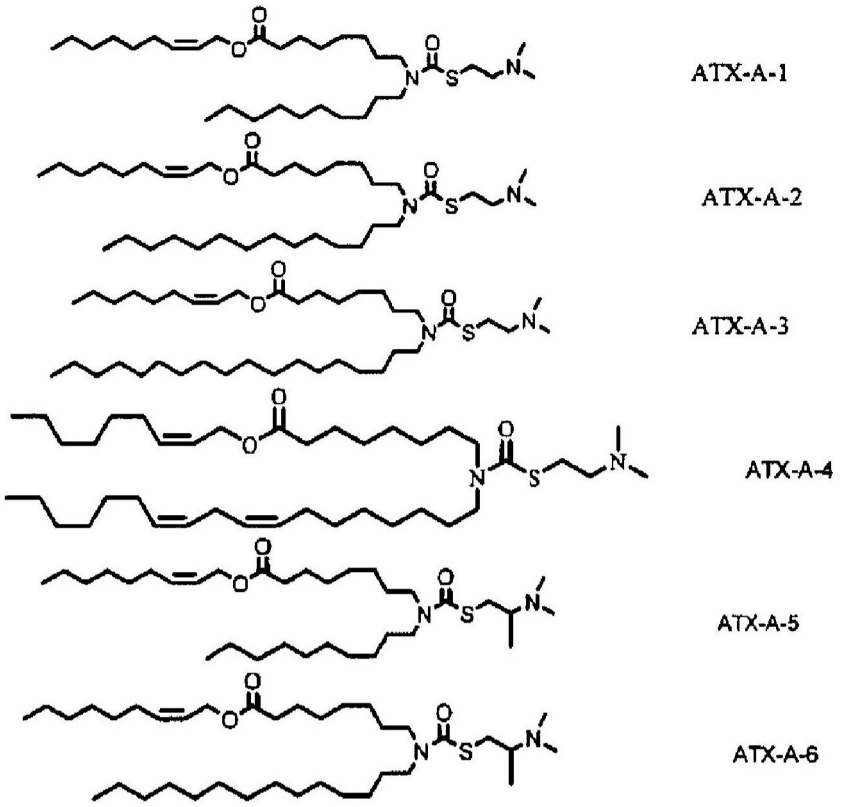
[0219]



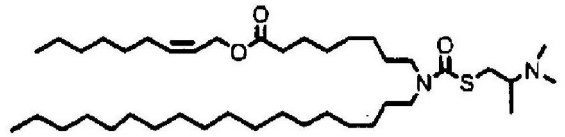
[0220] 示例性式V化合物提供于表4中。

[0221] 表4

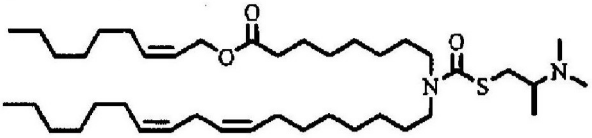
[0222]



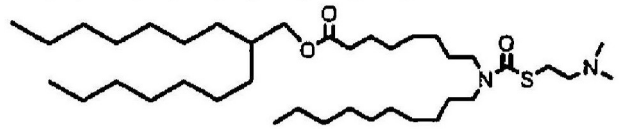
[0223]



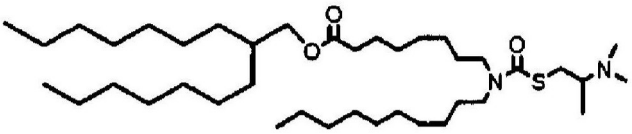
ATX-A-7



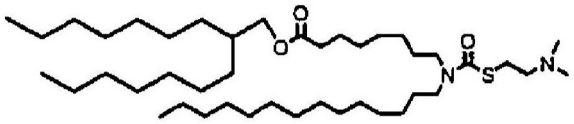
ATX-A-8



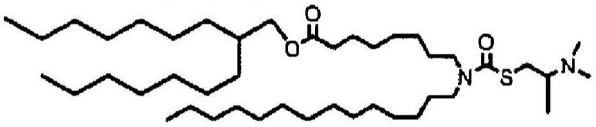
ATX-A-9



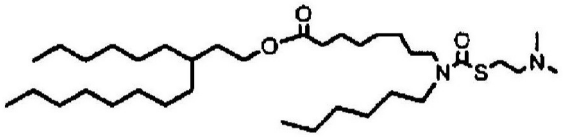
ATX-A-10



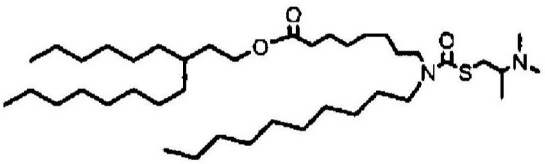
ATX-A-11



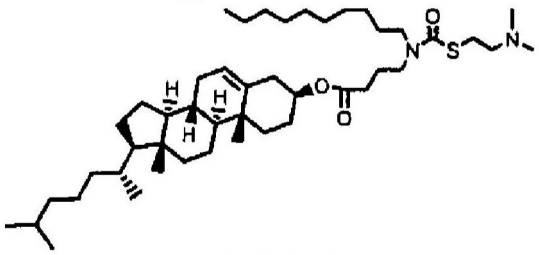
ATX-A-12



ATX-A-13



ATX-A-14



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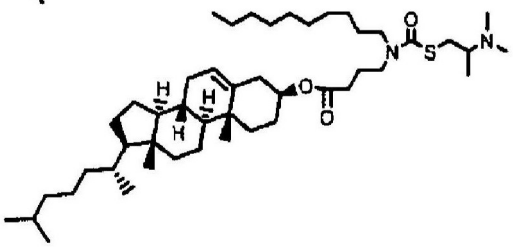
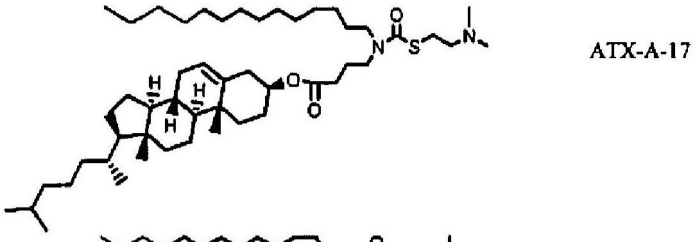


ATX-A-16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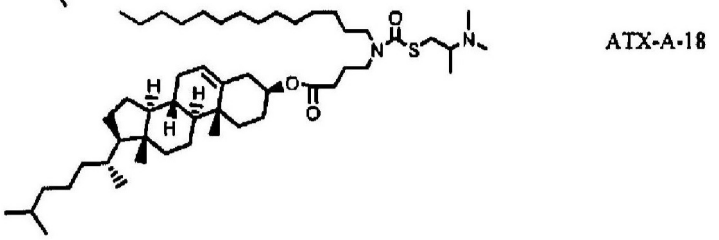
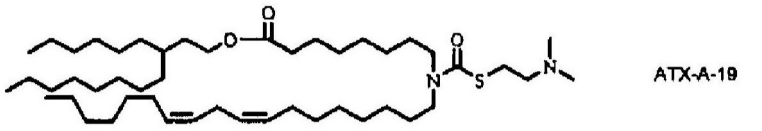
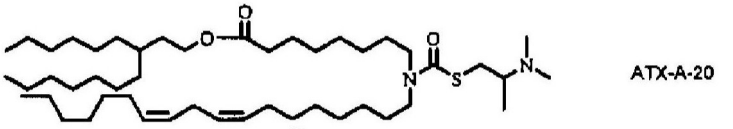

ATX-A-18

[0224]


ATX-A-19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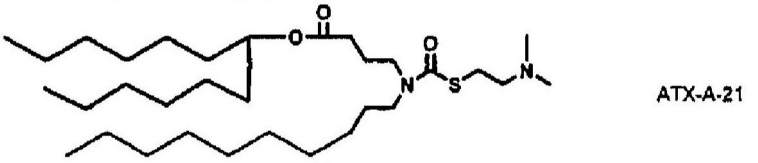
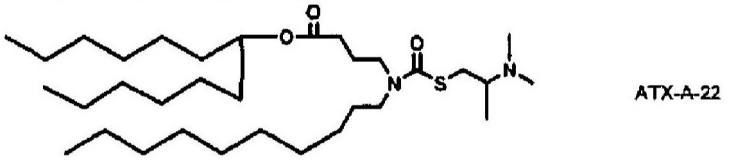
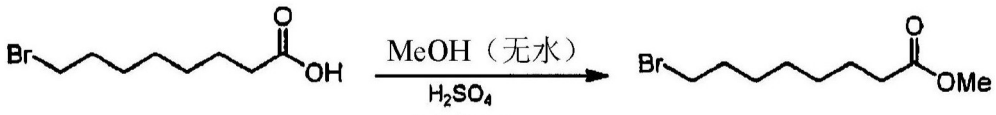

ATX-A-21


ATX-A-22

[0225] 表1示出了每种化合物的名称和结构、其分子量、其pKa和其在下文实例19中所述的分析法中的敲低生物活性(KD)。

[0226] 实例2．8-溴辛酸甲酯的合成

[0227]

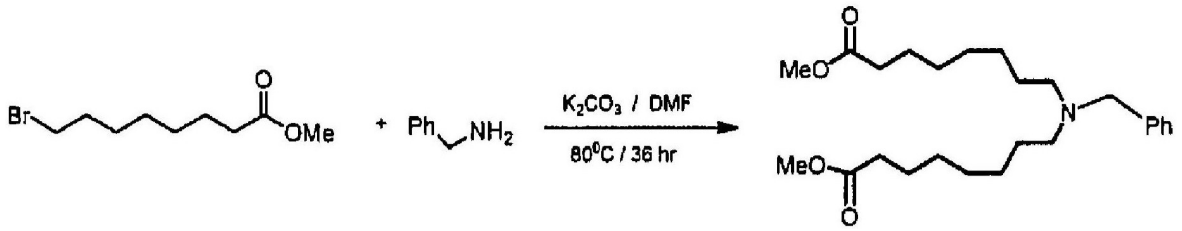


[0228] 在N_2气氛下，将8-溴辛酸溶解于无水甲醇中。逐滴添加浓H_2SO_4并且回流搅拌反应混合物三小时。

[0229] 通过薄层色谱监测反应，直到完成为止。在真空下完全去除溶剂。将反应混合物用乙酸乙酯稀释并且用水洗涤。用乙酸乙酯再萃取水层。用饱和$NaHCO_3$溶液洗涤总有机层。有机层再用水洗涤并最后用盐水洗涤。产物经无水Na_2SO_4干燥并浓缩。

[0230] 实例3．8,8′-(苯二基)二辛酸二甲酯的合成

[0231]



[0232] 获取无水K_2CO_3并且在N_2下添加至无水二甲基甲酰胺中。缓慢添加含苯甲胺的二甲基甲酰胺。随后在室温下添加溶解于二甲基甲酰胺中的8-溴辛酸甲酯。将反应混合物加热至80℃并在搅拌下维持反应36小时。

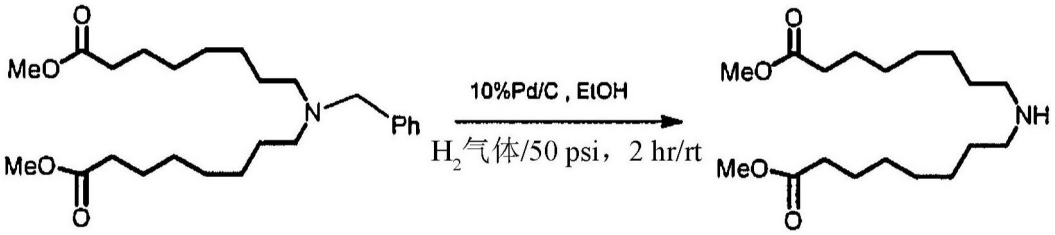
[0233] 通过薄层色谱监测反应，直到完成为止。将反应产物冷却到室温并且添加水。用乙酸乙酯萃取化合物。用乙酸乙酯再萃取水层。总有机层用水洗涤并最后用盐水溶液洗涤。产物经无水Na_2SO_4干燥并浓缩。

[0234] 通过硅胶柱色谱在3％甲醇/氯仿中纯化反应产物，回收44g纯产物。

[0235] 使用10％甲醇/氯仿的TLC系统，通过在茚三酮中炭化观测产物以Rf:0.8迁移。总产率为82％。化合物为浅棕色液体。通过^{1}H-NMR确认结构。

[0236] 实例4．8,8′-氮烷二基二辛酸二甲酯的合成

[0237]



[0238] 将8,8′-(苯二基)二辛酸二甲酯转移至氢化玻璃容器，并添加乙醇，接着添加10％Pd/C。将反应混合物在帕尔振荡器设备中在50磅/平方英寸[psi]H_2气氛压力下在室温下振荡两小时。

[0239] 反应产物经由硅藻土过滤并且用热乙酸乙酯洗涤。真空浓缩滤液。

[0240] 实例5．8,8′-((叔丁氧基羰基)氮烷二基)二辛酸二甲酯的合成

[0241]

[0242] 将8,8′-氮烷二基二辛酸二甲酯转移至DCM，将Et_3N添加至反应物质并冷却至0℃。将稀释于DCM中的Boc酸酐滴加至以上反应物。在添加完成之后，在室温下搅拌反应混合物三小时。

[0243] 用水淬灭反应物并分离DCM层。用DCM再萃取水相，合并的DCM层用盐水溶液洗涤并用Na_2SO_4干燥。在浓缩之后，收集40g粗化合物。

[0244] 通过柱色谱使用0-12％乙酸乙酯/己烷纯化粗反应产物。回收产率为48％。在用茚三酮炭化的情况下，单一产物通过薄层色谱在20％乙酸乙酯/己烷中以0.5的Rf迁移。

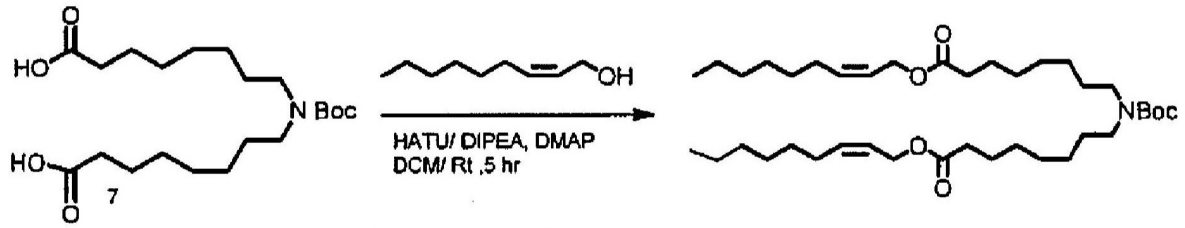
[0245] 实例6．8,8′-((叔丁氧羰基)氮烷二基)二辛酸的合成

[0246] 

[0247] 将8,8′-((叔丁氧羰基)氮烷二基)二辛酸二甲酯转移至THF。在室温下添加6N氢氧化钠溶液。在室温下在搅拌下维持反应过夜。

[0248] 在25℃下真空蒸发反应物质以去除THF。用5N HCl酸化反应产物。将乙酸乙酯添加至水层中。用水洗涤分离的有机层并用乙酸乙酯再萃取水层。合并的有机层用盐水溶液洗涤并经无水Na_2SO_4干燥。溶液浓缩得到18g粗物质。

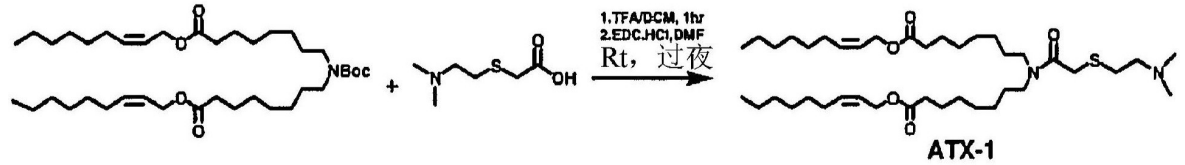
[0249] 实例7. 8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯的合成

[0250] 

[0251] 将8,8′-((叔丁氧羰基)氮烷二基)二辛酸溶解于无水DCM中。将HATU添加至此溶液中。在室温下将二异丙基乙胺缓慢添加至反应混合物。内部温度升高至40℃并且形成浅黄色溶液。将DMAP添加至反应混合物中,接着添加含顺式-2-壬烯-1-醇溶液的无水DCM。反应物变成棕色。在室温下搅拌反应物五小时。

[0252] 通过薄层色谱检查反应完全。将水添加至反应产物中,用DCM萃取。DCM层用水洗涤,接着用盐水溶液洗涤。有机层经无水Na_2SO_4干燥并浓缩,获得35g粗化合物。

[0253] 实例8.ATX-001的合成

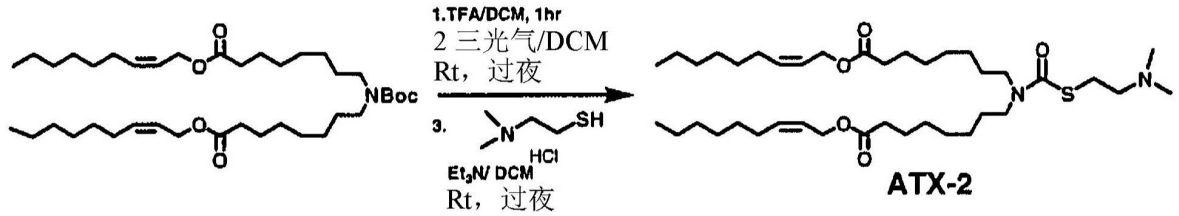
[0254] 

[0255] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(0.023mol,15g)溶解于无水二氯甲烷(DCM)(200ml)中。在0℃下添加三氟乙酸(TFA)以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并浓缩。在氮气下,将收集的粗产物(12g)溶解于无水DCM(85ml)中。添加三光气,将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。通过在N_2下蒸馏从反应物质去除DCM溶剂。将反应产物冷却至0℃,用DCM(50ml)、2-((2-(二甲基氨基)乙基)硫基)乙酸(0.039mol,6.4g)和碳化二亚胺(EDC·HCl)(0.054mol,10.4g)稀释。随后在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。用0.3M HCl溶液(75ml)稀释反应产物,并分离有机层。用DCM再萃取水层,合并的有机层用10%K_2CO_3水溶液(75ml)洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到10g粗物质。通过

硅胶柱(100-200目)使用3%MeOH/DCM纯化粗化合物。产量为10.5g(68%)。

[0256] 实例9.ATX-002的合成

[0257]



[0258] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(13.85mmol,9g)溶解于无水DCM(150ml)中。在0℃下添加TFA以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并浓缩。在氮气下,将收集的粗产物溶解于无水DCM(85ml)中。添加三光气,将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。通过在N_2下蒸馏从反应物质去除DCM溶剂。将反应产物冷却至0℃,用DCM(50ml)稀释并添加2-(二甲氨基)乙硫醇HCl(0.063mol,8.3g),接着添加Et_3N(无水)。随后在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。用0.3M HCl溶液(75ml)稀释反应产物,并分离有机层。用DCM再萃取水层,合并的有机层用10%K_2CO_3水溶液(75ml)洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到10g粗物质。通过硅胶柱(100-200目)使用3%MeOH/DCM纯化粗化合物。产量为3.1g。

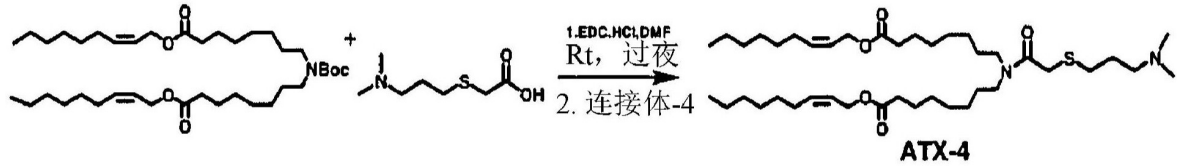
[0259] 实例10.ATX-003的合成

[0260]

[0261] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(0.00337mol,2.2g)溶解于无水DCM(20ml)中。在0℃下添加TFA以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并减压浓缩。在氮气下,将收集的粗产物溶解于无水DCM(10ml)中。添加三光气(0.0182mol,5.4g),将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。通过在N_2下蒸馏从反应物质去除DCM溶剂。将反应产物冷却至0℃,用DCM(15ml)稀释并添加2-(二甲氨基)丙硫醇HCl(0.0182mol,2.82g),接着添加Et_3N(无水)。随后在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。用0.3M HCl水溶液(20ml)稀释反应产物,并分离有机层。用DCM再萃取水层,合并的有机层用10%K_2CO_3水溶液(50ml)洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到5g粗物质。通过硅胶柱(100-200目)使用3%MeOH/DCM纯化粗化合物。产量为0.9g。

[0262] 实例11.ATX-004的合成

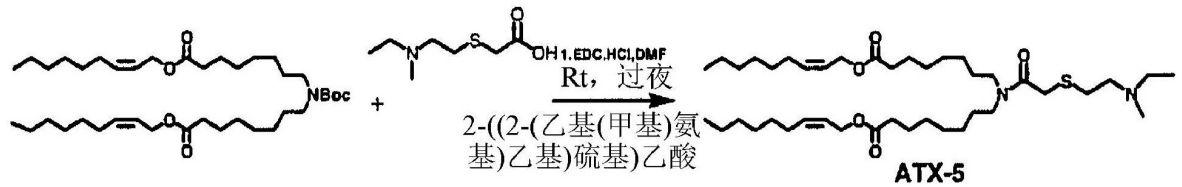
[0263]



[0264] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(0.023mol，15g)溶解于DCM(200ml)中。在0℃下添加TFA以引发反应。在搅拌下，使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物，粗残余物用DCM稀释并用10％$NaHCO_3$溶液洗涤。用DCM再萃取水层，合并的有机层用盐水溶液洗涤，经Na_2SO_4干燥并浓缩。在氮气下，将收集的粗产物8,8′-氮烷二基二辛酸二((Z)-壬-2-烯-1-基)酯(5.853mmol，3.2g)溶解于无水二甲基甲酰胺(DMF)中，并添加2-((3-(二甲氨基)丙基)硫基)乙酸(10.48mmol，1.85g)和EDC·HCl(14.56mmol，2.78g)。在室温下搅拌反应混合物过夜。将反应物用水(30ml)淬灭并用DCM(30ml)稀释，并且分离有机层。用DCM再萃取水层，合并的有机层用10％K_2CO_3水溶液洗涤并经无水Na_2SO_4干燥。通过硅胶柱(100-200目)使用3％MeOH/DCM纯化粗化合物。产量为1g(24.2％)。

[0265] 实例12.ATX-005的合成

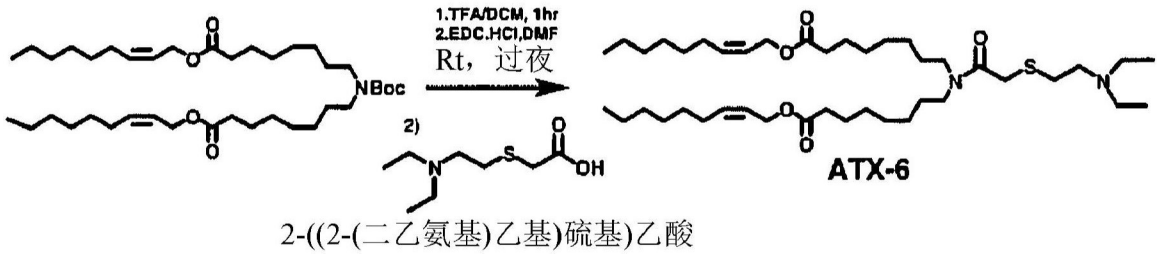
[0266]



[0267] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(0.023mol，15g)溶解于无水DCM(200ml)中。在0℃下添加TFA以引发反应。在搅拌下，使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物，粗残余物用DCM稀释并用10％$NaHCO_3$溶液洗涤。用DCM再萃取水层，合并的有机层用盐水溶液洗涤，经Na_2SO_4干燥并浓缩。在氮气下，将粗反应产物8,8′-氮烷二基二辛酸二((Z)-壬-2-烯-1-基)酯(5.853mmol，3.2g)溶解于二甲基甲酰胺(DMF)中。添加2-((3-(二甲氨基)丙基)硫基)乙酸(10.48mmol，1.85g)和EDC·HCl(14.56mmol，2.78g)并在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。将反应产物用水(30ml)淬灭并用DCM(30ml)稀释。用DCM再萃取水层，合并的有机层用10％K_2CO_3水溶液(75ml)洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到5g粗物质。通过硅胶柱(100-200目)使用3％MeOH/DCM纯化粗化合物。产量为1g(24.2％)。

[0268] 实例13.ATX-006的合成

[0269]

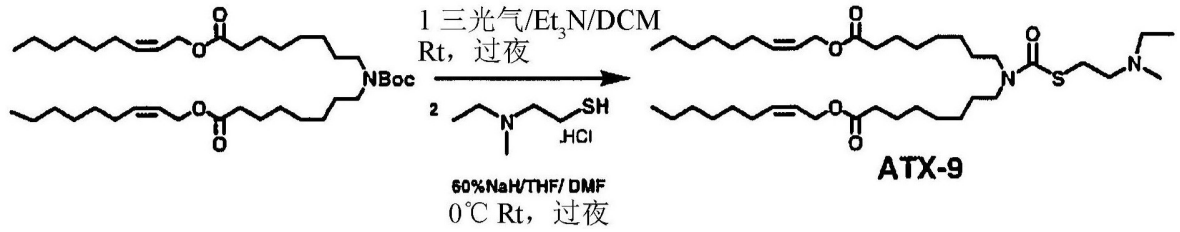


2-((2-(二乙氨基)乙基)硫基)乙酸

[0270] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯溶解于无水DCM(150ml)中。在0℃下添加TFA以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并浓缩。在氮气下,将收集的粗产物溶解于无水DCM(85ml)中。添加三光气,将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。在氮气气氛下将粗反应产物溶解于无水DMF中,并添加2-((2-(二乙氨基)乙基)硫基)乙酸(3.93mmol,751mg)和EDC·HCl(5.45mmol,1.0g)。在室温下搅拌反应混合物过夜。用水(3ml)淬灭反应并在25℃下真空去除过量DMF。用水稀释反应产物并用DCM(20ml)萃取水层三次。合并的有机层用盐水溶液洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到2g粗物质。在通过硅胶柱(100-200目)使用3%MeOH/DCM纯化之后,产量为1.2g(76%)。

[0271] 实例14.ATX-009的合成

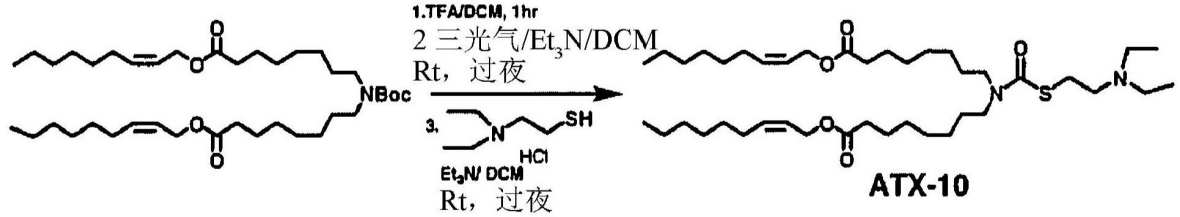
[0272]



[0273] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(13.85mmol,9g)溶解于无水DCM(20ml)中。在0℃下添加TFA以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并浓缩。在氮气气氛下,将8,8′-氮烷二基二辛酸二((Z)-壬-2-烯-1-基)酯(0.909mmol,500mg)溶解于无水DCM(20ml)中。添加三光气,将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。通过在氮气气氛下蒸馏从反应物质去除DCM溶剂。将2-(乙基(甲基)氨基)乙-1-硫醇盐酸盐(4.575mmol,715mg)溶解于DMF(7ml)中,并在0℃下将四氢呋喃(THF)(5ml)逐滴添加至含氢化钠悬浮液的THF中。随后在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。用乙酸乙酯和冷水稀释反应产物。用5%HCl(9ml)中和反应物并分离有机层。用乙酸乙酯(EtOAc)(20ml)再萃取水层,在冷水和盐水中洗涤,将合并的有机层洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到1g粗产物。通过硅胶柱(100-200目)使用3%MeOH/DCM纯化化合物,得到100mg。

[0274] 实例15.ATX-010的合成

[0275]

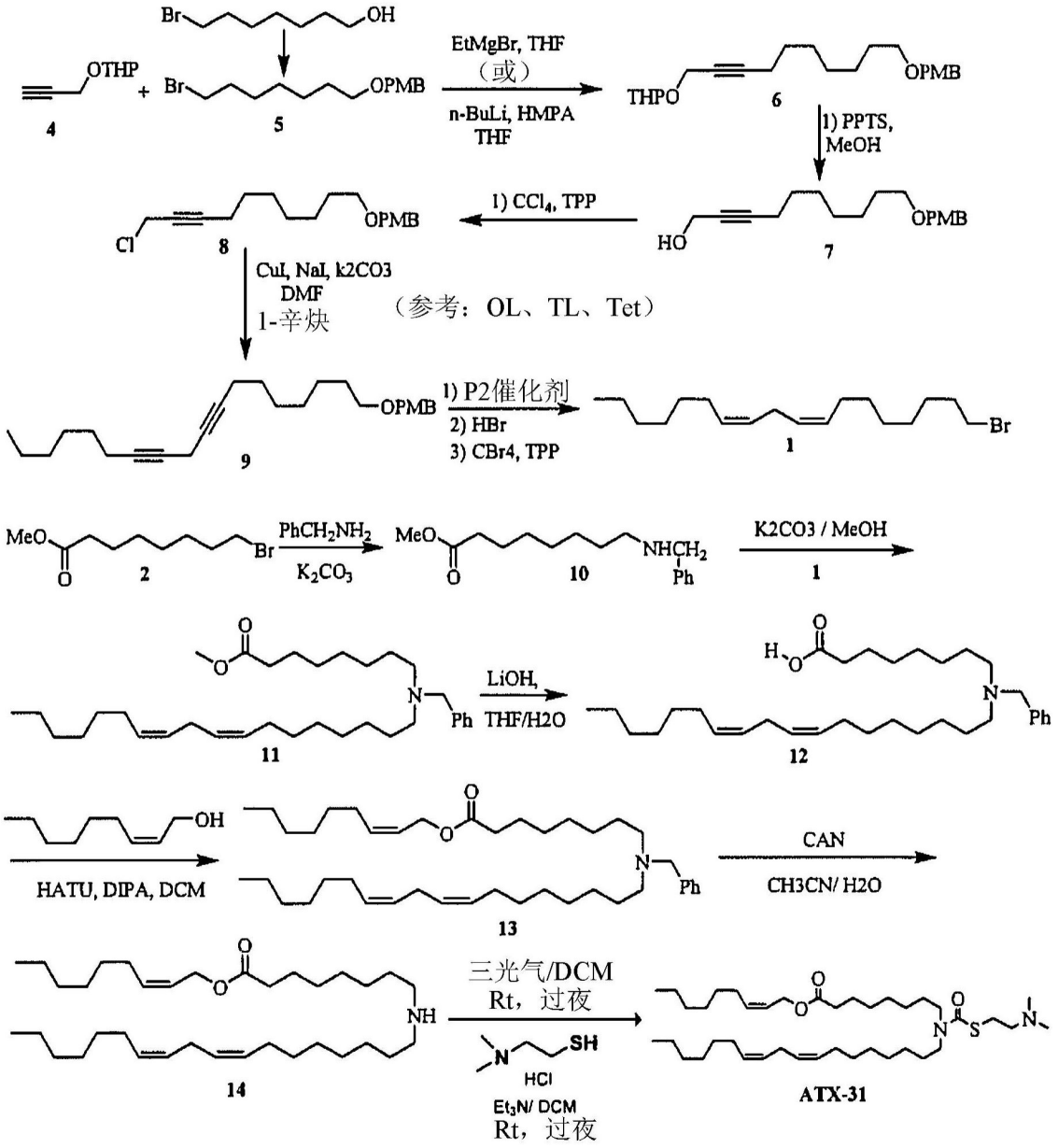


[0276] 将8,8′((叔丁氧羰基)氮烷二基)二辛酸二((Z)-壬-2-烯-1-基)酯(3.079mmol,

2g)溶解于无水DCM(20ml)中。在0℃下添加TFA以引发反应。在搅拌下,使反应温度缓慢升温至室温持续30分钟。薄层色谱显示反应完成。在40℃下真空浓缩反应产物,粗残余物用DCM稀释并用10%$NaHCO_3$溶液洗涤。用DCM再萃取水层,合并的有机层用盐水溶液洗涤,经Na_2SO_4干燥并浓缩。在氮气下,将收集的粗产物溶解于无水DCM(20ml)中。添加三光气(14.55mmol,4.32g),将反应混合物冷却至0℃并逐滴添加Et_3N。在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。通过在N_2下蒸馏从反应物质去除DCM溶剂。将反应产物冷却至0℃,用DCM(20ml)稀释并添加2-(二甲氨基)乙硫醇HCl(0.063mol,8.3g),接着添加Et_3N(无水)。随后在室温下搅拌反应混合物过夜。薄层色谱显示反应完成。用0.3M HCl溶液(20ml)稀释反应产物,并分离有机层。用DCM再萃取水层,合并的有机层用10%K_2CO_3水溶液(20ml)洗涤并经无水Na_2SO_4干燥。浓缩溶剂得到10g粗物质。通过硅胶柱(100-200目)使用3%MeOH/DCM纯化粗化合物。产量为1.4g(75%)。

[0277] 实例16.ATX-A-4(下文称为ATX-031)的合成

[0278]



[0279] 实例17.来自表1的ATX-011至ATX-017、ATX-021至ATX-023和ATX-026至ATX-030以及表2、3和4的化合物的合成

[0280] ATX-011至ATX-017、ATX-021至ATX-023和ATX-026至ATX-030、ATX-A-1至ATX-A-22以及表2、3和4的化合物的合成按照实例1-15的合成，取代其中所描述的合成反应的适当起始成分。

[0281] 实例18.活体内小鼠因子VII沉默

[0282] 使用肝脏定向的脂质体文库的活体内筛选，测试促成肝细胞(所述细胞构成肝实质)中高水平siRNA介导的基因沉默的一系列化合物。因子VII(一种凝血因子)是适用于分析功能性siRNA递送至肝脏的目标基因。因为这种因子是在肝细胞中特定产生，所以基因沉默指示成功递送至实质，与递送至网状内皮系统的细胞(例如库普弗细胞(Kupffer cell))相反。此外，因子VII是可易于在血清中测量的分泌性蛋白，从而无需对动物进行安乐死。在mRNA水平下的沉默可易于通过测量蛋白质水平来测定。这是因为蛋白质的半衰期短(2-5小时)。C57BL/6小鼠(查尔斯河实验室(Charles River Labs))经由尾静脉注射接受0.006ml/g体积生理盐水或含siRNA的脂质体配制品。在投与之后48小时，动物通过吸入异氟醚麻醉并通过眶后放血而将血液采集至血清分离管中。根据制造商的方案，使用生色分析法(Biophen FVII，安尼亚拉公司(Aniara Corporation))测定样品中因子VII蛋白的血清水平。使用从生理盐水处理的动物采集的血清生成标准曲线。

[0283] 针对因子VII的具有siRNA的组合物是用ATX-001、ATX-002、ATX-003和ATX-547以及比较样品NC1和MC3(Alnylam)配制。将这些以0.3mg/kg和1mg/kg注射至动物中。在向C57BL6小鼠投与siRNA配制品之后，测量由MC3(0.3mg/kg)、NC1(0.3mg/kg)、ATX-547(0.3mg/kg)、ATX-001(0.3和1.0mg/kg)、ATX-002(0.3和1.0mg/kg)和ATX-003(0.3和1.0mg/kg)囊封的siRNA敲低小鼠血浆中的因子VII的能力。结果显示ATX-001和ATX-002与对照物相比在0.3mg/kg下最有效(图1和2)。

[0284] 在向C57BL6小鼠投与siRNA配制品之后，测量由MC3(0.3和1.5mg/kg)、NC1(0.3mg/kg)、ATX-547(0.1和0.3mg/kg)、ATX-004(0.3)、ATX-006(0.3和1.0mg/kg)、ATX-010(0.3mg/kg)和ATX-001(0.3和1.5mg/kg)囊封的siRNA敲低小鼠血浆中的因子VII。结果显示ATX-001和ATX-010最有效(图3和4)。0.3mg/kg或0.05mg/kg ATX-018、ATX-019和ATX-020显示示例性化合物的敲低活性(表1)。

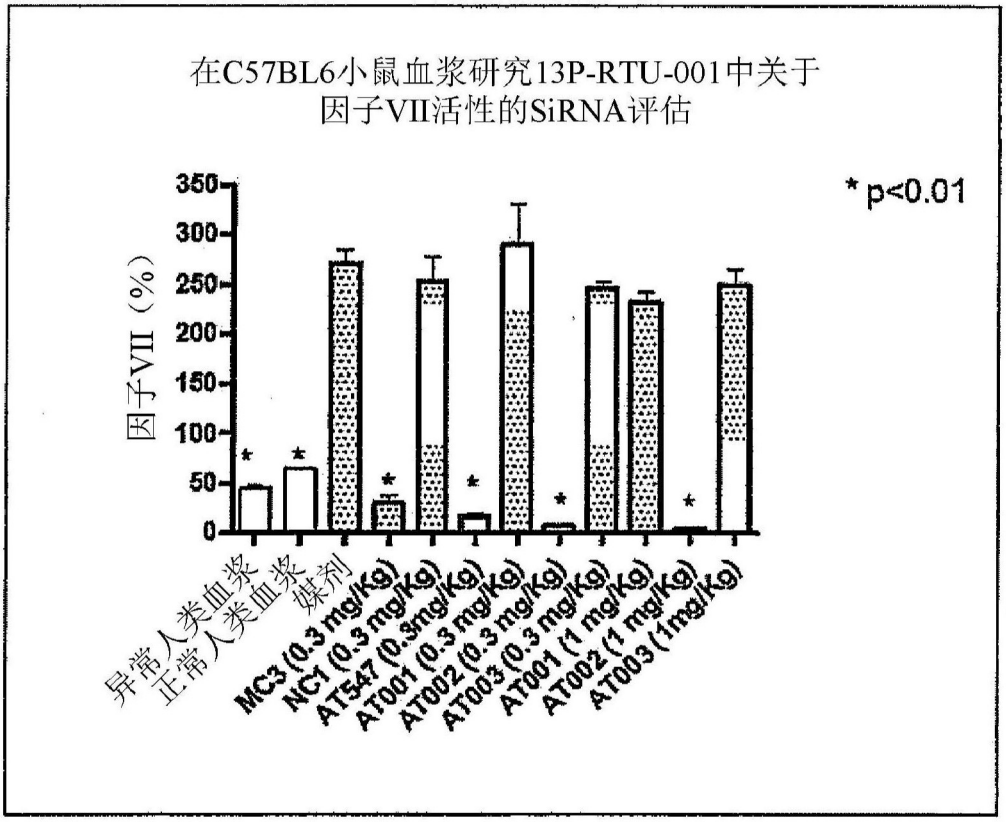

在C57BL6小鼠血浆研究13P-RTU-001中关于
因子VII活性的SiRNA评估
* p<0.01
因子VII（%）
350
300
250
200
150
100
50
0
异常人类血浆
正常人类血浆
媒剂
MC3 (0.3 mg/kg)
NC1 (0.3 mg/kg)
AT547 (0.3mg/kg)
AT001 (0.3 mg/kg)
AT002 (0.3 mg/kg)
AT003 (0.3 mg/kg)
AT001 (1 mg/kg)
AT002 (1 mg/kg)
AT003 (1mg/kg)

图1

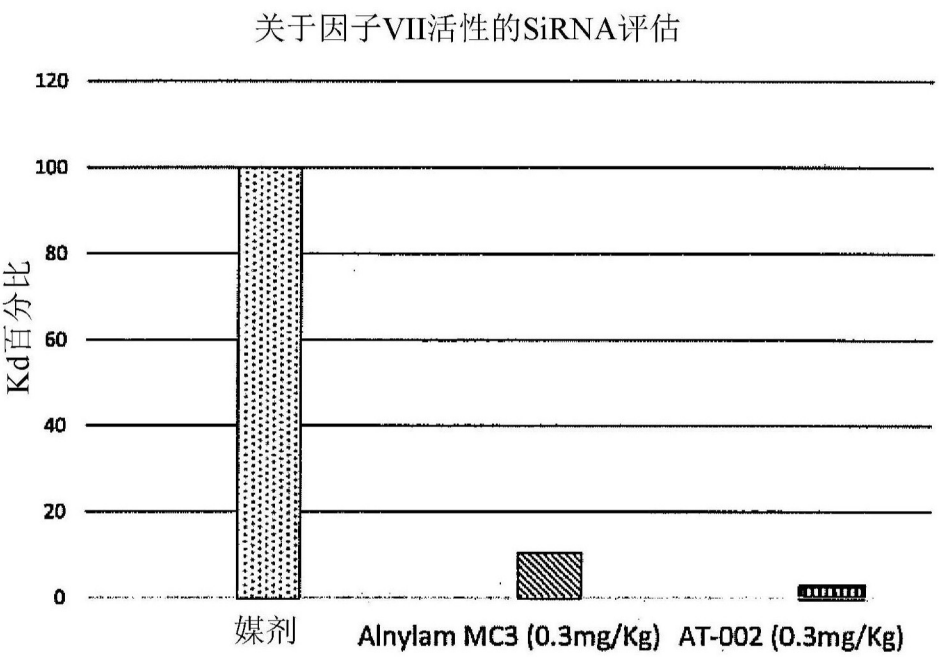

关于因子VII活性的SiRNA评估
Kd百分比
120
100
80
60
40
20
0
媒剂
Alnylam MC3 (0.3mg/Kg)
AT-002 (0.3mg/Kg)

图2

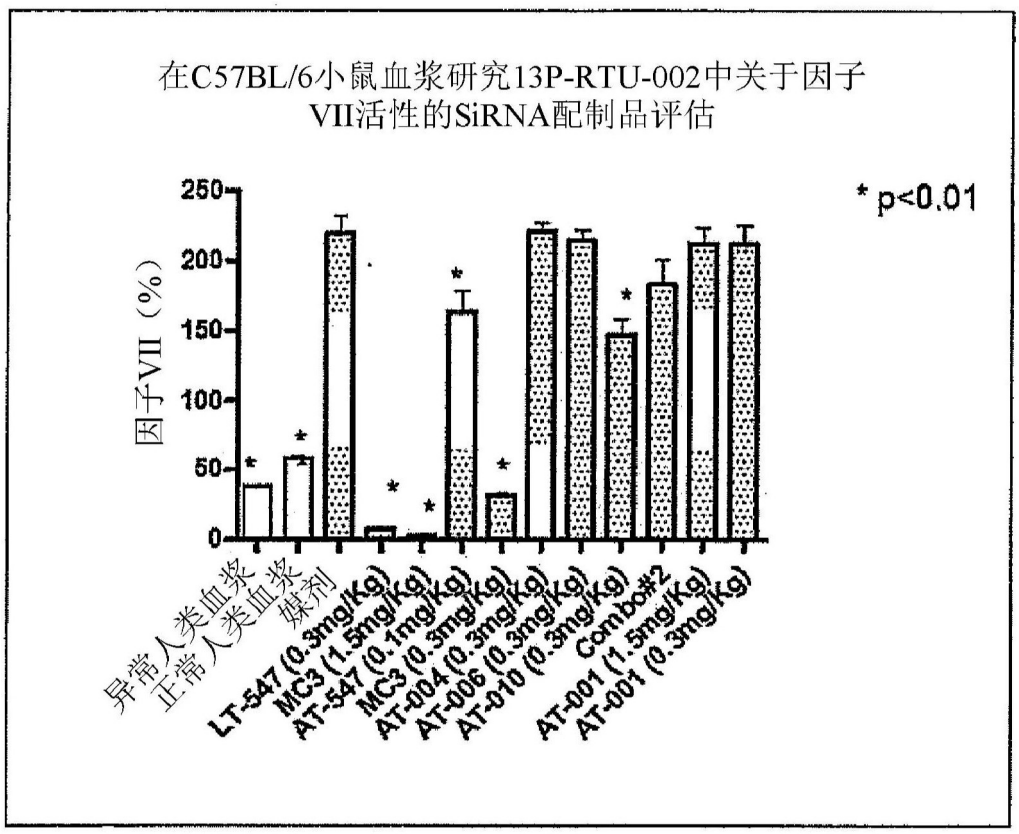

在C57BL/6小鼠血浆研究13P-RTU-002中关于因子VII活性的SiRNA配制品评估
* p<0.01
因子VII（%）
250
200
150
100
50
0
异常人类血浆
正常人类血浆
媒剂
LT-547 (0.3mg/Kg)
MC3 (1.5mg/Kg)
AT-547 (0.1mg/Kg)
MC3 (0.3mg/Kg)
AT-004 (0.3mg/Kg)
AT-006 (0.3mg/Kg)
AT-010 (0.3mg/Kg)
Combo#2
AT-001 (1.5mg/Kg)
AT-001 (0.3mg/Kg)

图3

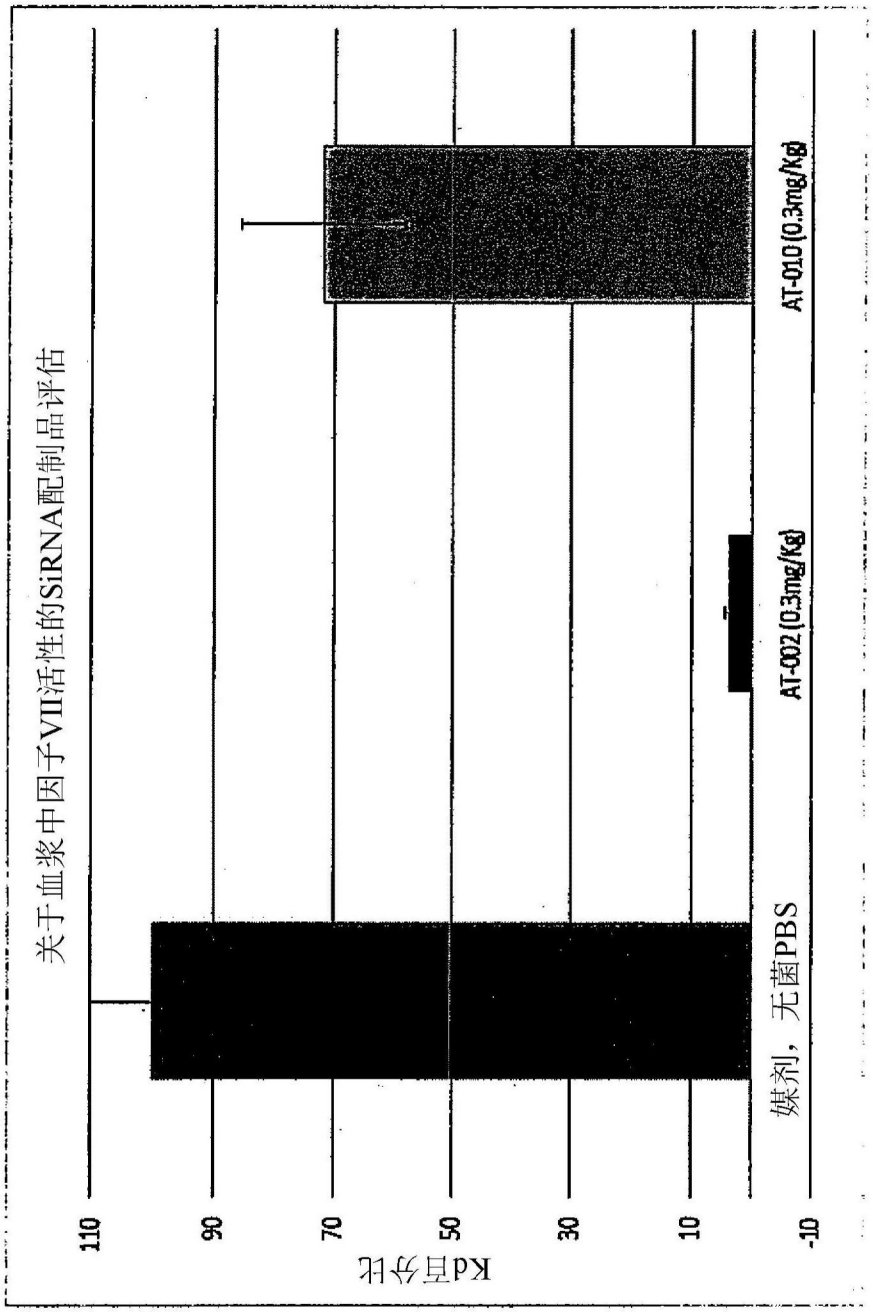
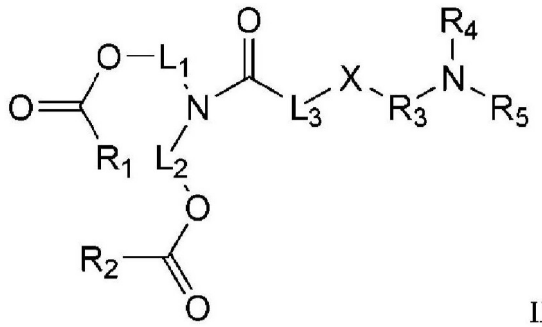

关于血浆中因子VII活性的SiRNA配制品评估
Kd百分比
110
90
70
50
30
10
-10
媒剂，无菌PBS
AT-002 (0.3mg/Kg)
AT-010 (0.3mg/Kg)

图4

1.一种式II化合物，



II

其中

R_1和R_2都由1至14个碳所组成的直链烷基、或2至14个碳所组成的烯基或炔基组成；

L_1和L_2都由5至18个碳所组成的直链亚烷基或亚烯基组成，或与N一起形成杂环；

X是S；

L_3由键或1至6个碳所组成的直链亚烷基组成，或与N一起形成杂环；

R_3由1至6个碳所组成的直链或支链亚烷基组成；以及

R_4和R_5是相同或不同的，各自由氢或1至6个碳所组成的直链或支链烷基组成；

或其药学上可接受的盐。

2.根据权利要求1所述的化合物，其中R_1和R_2都是烯基。

3.根据权利要求1所述的化合物，其中R_1和R_2都是烷基。

4.根据权利要求1所述的化合物，其中L_1和L_2都是直链亚烷基。

5.根据权利要求1所述的化合物，其中L_1和L_2都是亚烯基。

6.根据权利要求1所述的化合物，其中L_1和L_2都由五个碳所组成的直链亚烷基组成。

7.根据权利要求1所述的化合物，其中R_3由亚乙基或亚丙基组成。

8.根据权利要求1所述的化合物，其中R_4和R_5各自由氢、甲基或乙基组成。

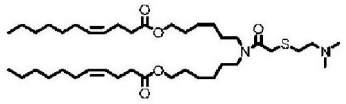
9.根据权利要求1所述的化合物，其中L_3由键组成。

10.根据权利要求1所述的化合物，其中L_3由直链亚烷基组成。

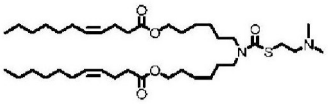
11.根据权利要求1所述的化合物，其中R_1和R_2都由十个碳所组成的直链烯基组成。

12.根据权利要求1所述的化合物，其选自式1)至12)的化合物：

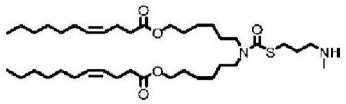
1)



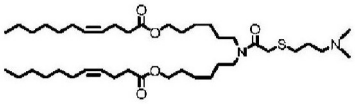
2)



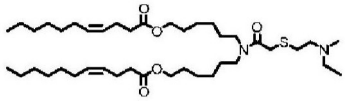
3)



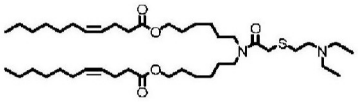
4)



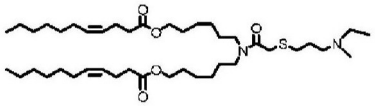
5)



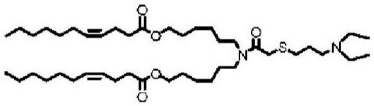
6)



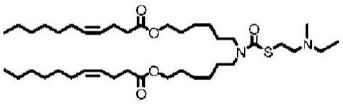
7)



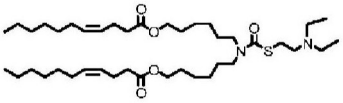
8)



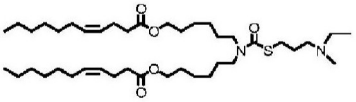
9)



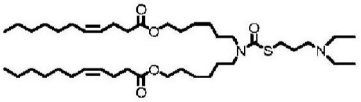
10)



11)

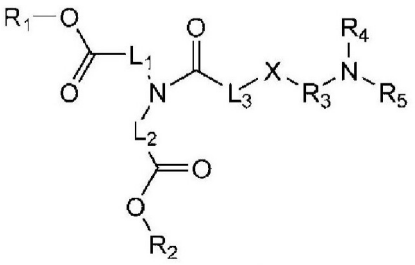


12)

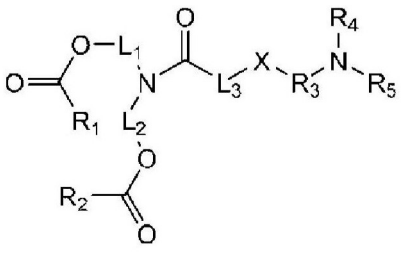


。

13.一种式III或IV化合物，



III



IV

其中

R_1由具有12至20个碳的支链烷基组成，

R_2由具有5至10个碳的直链烷基或具有12至20个碳的支链烷基组成，

L_1和L_2各自由键或具有1至3个碳原子的直链烷基组成，

X由S或O组成，

L_3由键或1至6个碳所组成的亚烷基组成，

R_3由1至6个碳所组成的直链或支链亚烷基组成，以及

R_4和R_5是相同或不同的，各自由1至6个碳所组成的直链或支链烷基组成；

或其药学上可接受的盐。

14.根据权利要求13所述的化合物，其中L_3由键组成。

15.根据权利要求17所述的化合物，其中L_3由亚烷基组成。

16.根据权利要求13所述的化合物，其中X由S组成。

17.根据权利要求13所述的化合物，其中R_3由亚乙基组成。

18.根据权利要求13所述的化合物，其中R_3由亚正丙基或亚异丙基组成。

19.根据权利要求13所述的化合物，其中R_4和R_5各自由甲基、乙基或异丙基组成。

20.根据权利要求13所述的化合物，其中L_1和L_2各自由键组成。

21.根据权利要求13所述的化合物，其中L_1和L_2各自由直链亚烷基组成。

22.根据权利要求13所述的化合物，其中L_1和L_2各自由亚甲基组成。

23.根据权利要求13所述的化合物，其中R_2由直链烷基组成。

24.根据权利要求13所述的化合物，其中R_2由支链烷基组成。

25.根据权利要求24所述的化合物，其中所述支链烷基由19或20个碳原子组成。

26.根据权利要求24所述的化合物，其中所述支链烷基由13或14个碳原子组成。

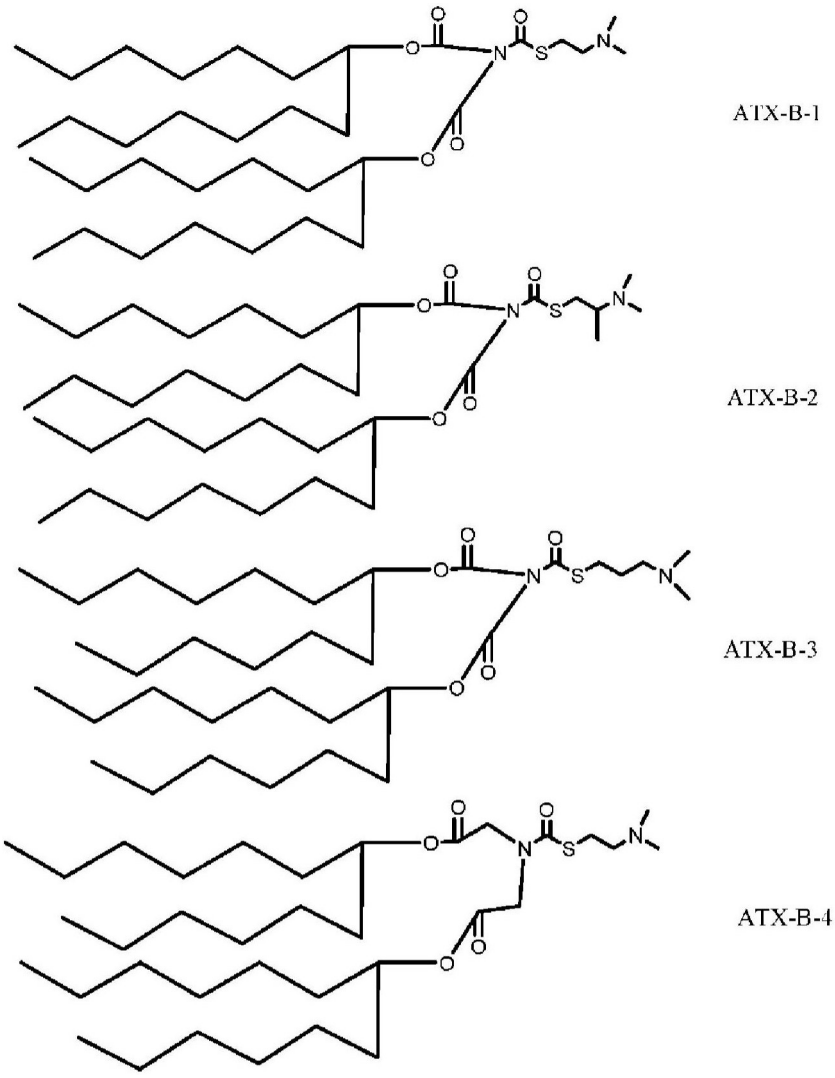
27.根据权利要求13所述的化合物，其中L_3由亚甲基组成，R_3由亚乙基组成，X_2由S组成，并且R_4和R_5都由甲基组成。

28.根据权利要求13所述的化合物，其中L_3由键组成，R_3由亚乙基组成，X由S组成，并且R_4和R_5都由甲基组成。

29.根据权利要求13所述的化合物，其中L_3由键组成，R_3由亚正丙基组成，X由S组成，并且R_4和R_5都由甲基组成。

30.根据权利要求13所述的化合物，其中L_3由键组成，R_3由亚异丙基组成，X由S组成，并且R_4和R_5都由甲基组成。

31.根据权利要求13所述的化合物，其选自式ATX-B-1至ATX-B-12的化合物：

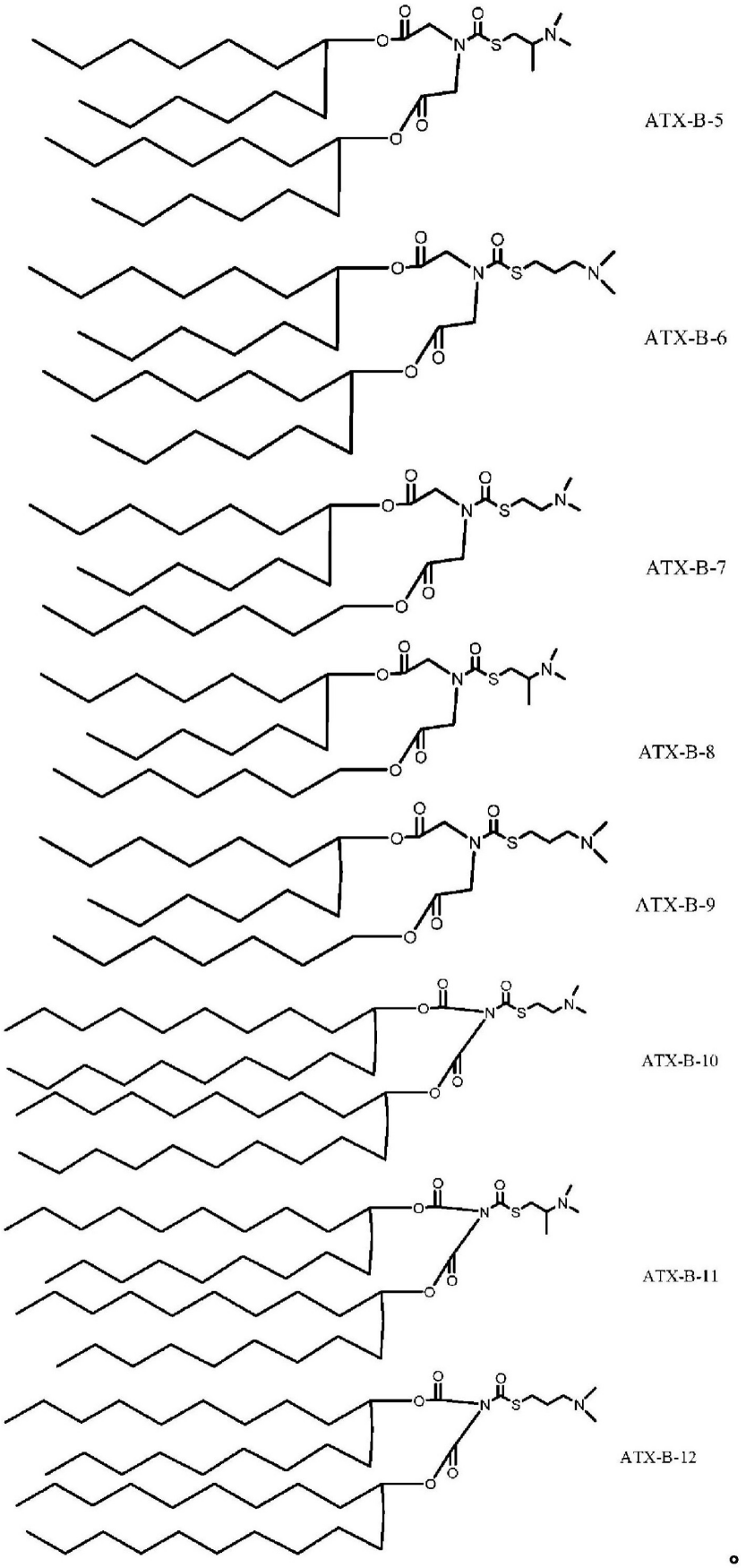


ATX-B-1

ATX-B-2

ATX-B-3

ATX-B-4



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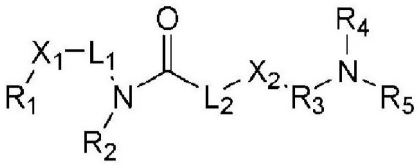
32.一种式V化合物，



V

其中

R_1由1至20个碳所组成的直链或支链烷基、或2至12个碳所组成的烯基或炔基、或胆固醇基组成；

R_2由1至20个碳所组成的直链或支链烷基或2至20个碳所组成的烯基组成；

L_1由3至9个碳所组成的直链烷基组成，或当R_1由胆固醇基组成时，L_1由3至4个碳所组成的直链亚烷基或烯基组成；

X_1由-O-(CO)-或-(CO)-O-组成；

X_2由S或O组成；

L_2由键或具有1至6个碳的直链亚烷基组成；

R_3由具有1至6个碳的直链或支链亚烷基组成；以及

R_4和R_5是相同或不同的，各自由具有1至6个碳的直链或支链烷基组成；

或其药学上可接受的盐。

33.根据权利要求32所述的化合物，其中X_1由-O-(CO)-组成。

34.根据权利要求32所述的化合物，其中X_2由S组成。

35.根据权利要求32所述的化合物，其中R_3由亚乙基组成。

36.根据权利要求32所述的化合物，其中R_3由亚正丙基或亚异丙基组成。

37.根据权利要求32所述的化合物，其中R_4和R_5各自由甲基、乙基或异丙基组成。

38.根据权利要求32所述的化合物，其中L_2由键组成。

39.根据权利要求32所述的化合物，其中L_2由亚甲基组成。

40.根据权利要求32所述的化合物，其中R_2由烷基组成。

41.根据权利要求32所述的化合物，其中R_1和R_2都由支链烷基组成。

42.根据权利要求41所述的化合物，其中所述支链烷基由19或20个碳原子组成。

43.根据权利要求41所述的化合物，其中所述支链烷基由13或14个碳原子组成。

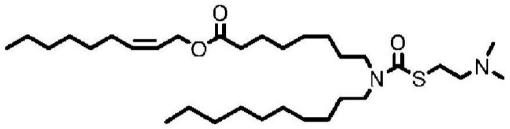
44.根据权利要求32所述的化合物，其中L_2由亚甲基组成，R_3由亚乙基组成，X_1由-O-(CO)-组成，X_2是S，并且R_4和R_5都由甲基组成。

45.根据权利要求32所述的化合物，其中L_2由键组成，R_3由亚乙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

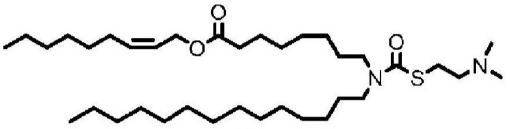
46.根据权利要求32所述的化合物，其中L_2由键组成，R_3由亚正丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

47.根据权利要求32所述的化合物，其中L_2由键组成，R_3由亚异丙基组成，X_1由-O-(CO)-组成，X_2由S组成，并且R_4和R_5都由甲基组成。

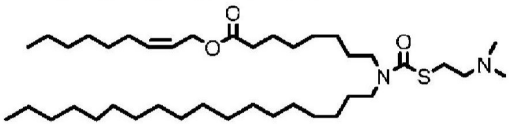
48.根据权利要求32所述的化合物，其选自由式ATX-A-1至ATX-A-22的化合物组成的群组：



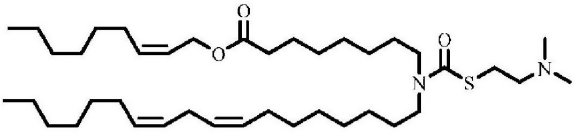
ATX-A-1



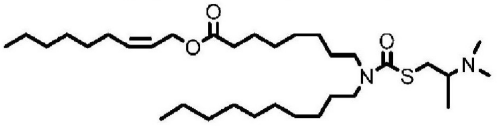
ATX-A-2



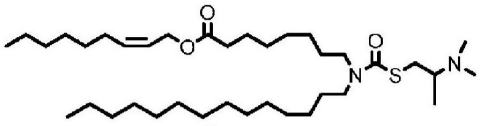
ATX-A-3



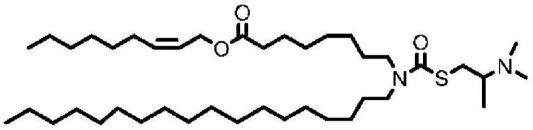
ATX-A-4



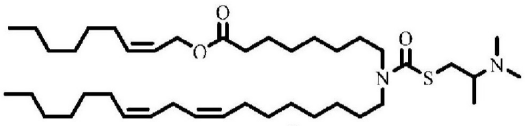
ATX-A-5



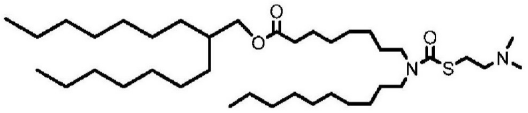
ATX-A-6



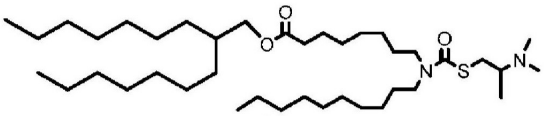
ATX-A-7



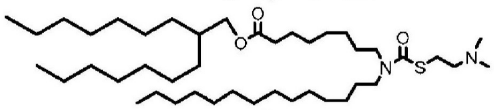
ATX-A-8



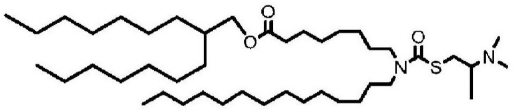
ATX-A-9



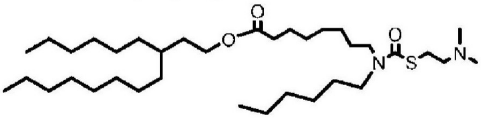
ATX-A-10



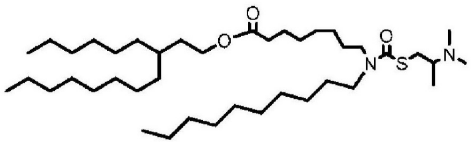
ATX-A-11



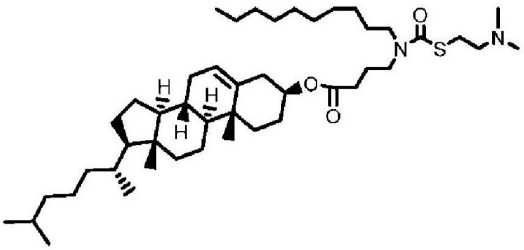
ATX-A-12



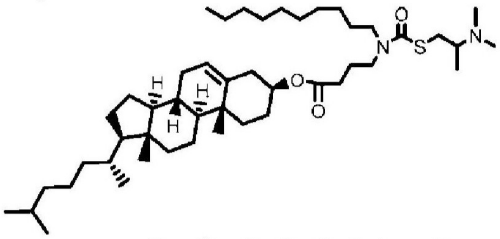
ATX-A-13



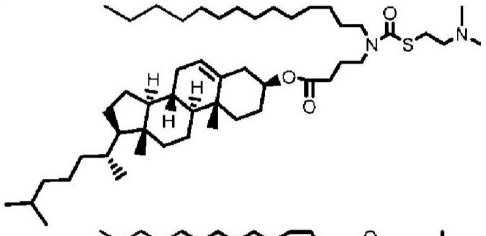
ATX-A-14



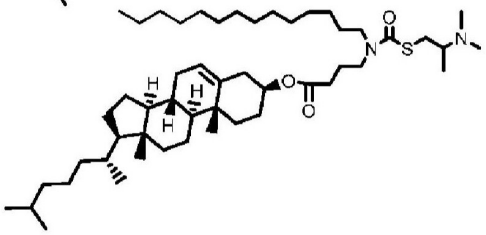
ATX-A-15



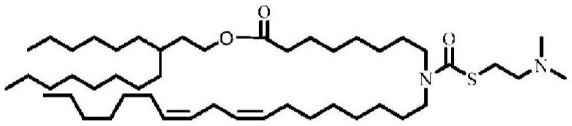
ATX-A-16



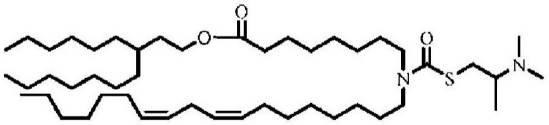
ATX-A-17



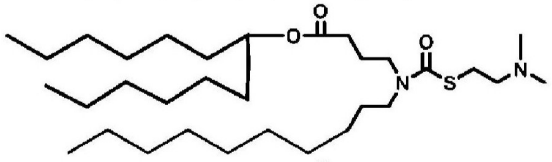
ATX-A-18



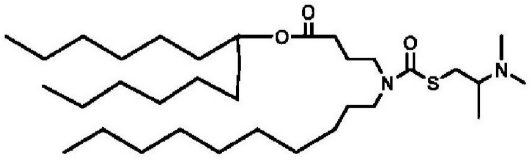
ATX-A-19



ATX-A-20



ATX-A-21



ATX-A-22

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