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(71) Applicant (for all designated States except US): **ABBOTT LABORATORIES** [US/US]; 100 Abbott Park Road, Abbott Park, Illinois 60064 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HANSEN, Todd M.** [US/US]; 191 Seymour Avenue, Grayslake, Illinois 60030 (US). **HUBBARD, Robert D.** [US/US]; 2796 Hancock Court, Lindenhurst, Illinois 60046 (US). **LI, Leiming** [CN/US]; 1166 Gail Drive, Buffalo Grove, Illinois 60089 (US). **TIAN, Lu** [US/US]; 1516 Meadow Drive, Blue Bell, Pennsylvania 19422 (US). **WADA, Carol K.** [US/US]; 819 Forest Avenue, Apt. 3W, Evanston, Illinois 60202 (US). **ZHAO, Xiaobin** [CN/US]; 10707 Great Arbor Drive, Potomac, Maryland 20854 (US).

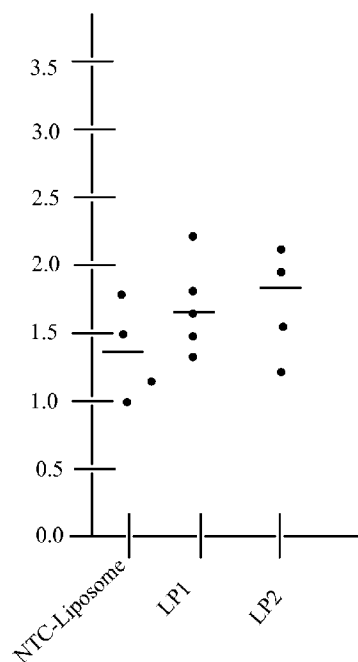
(74) Agents: **REININGER, Irene M.** et al.; Abbott Laboratories, Dept. 0377, Bldg. AP6A-1A, 100 Abbott Park Road, Abbott Park, Illinois 60064-6008 (US).

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(54) Title: POLYETHYLENE GLYCOL LIPID CONJUGATES AND USES THEREOF

FIGURE 1 illustrates in vivo activity of Lipid-Based Particle 1 (LP1) and Lipid-Based Particle 2 (LP2) versus a non-targeted composition (NTC).



(57) Abstract: Polyethylene glycol (PEG)-lipid conjugates, polyethylene glycol (PEG)-lipid conjugate based drug delivery systems, ways to make them and methods of treating diseases using them are disclosed.



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SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT,
TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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POLYETHYLENE GLYCOL LIPID CONJUGATES AND USES THEREOF

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FIELD OF THE INVENTION

This invention pertains to polyethylene glycol (PEG)-lipid conjugates, polyethylene glycol (PEG)-lipid conjugate based drug delivery systems, ways to make them, and methods of treating diseases using them.

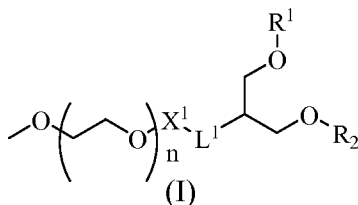
BACKGROUND OF THE INVENTION

Through the development of novel delivery formulations, research is now able to focus more on improving efficacy on the therapeutic and clinical efficacious of therapeutic agents such as nucleic acids, RNA, antisense oligonucleotide, a DNA, a plasmid, a ribosomal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), and small nuclear RNA (snRNA). Such novel delivery formulations will need, for example, to allow for appropriate internalization of the therapeutic agent into the cell, agents sufficient absorption from the site of administration, distribution to various tissues, sufficient residence time, concentration at the sites of action to elicit effective biologic response, while minimizing toxicity, in addition to also maintaining it's stability, and size. To this end, many efforts have been made to develop liposome or cationic polymer complexes with polyethylene glycol (PEG) or other neutral or targeting moieties. Ogris et al., *Gene Ther.* 6, 595-605 (1999).

However, many of the agents to date have not been found to successfully deliver therapeutic agents or to successfully deliver therapeutic agents while minimizing toxicity. As such, there is a clear need in the art to develop a novel delivery system with an improved toxicity profile as well as enhanced therapeutic agent efficacy.

SUMMARY OF THE INVENTION

One embodiment of this invention pertains to polyethylene glycol (PEG)-lipid conjugates, or mixtures thereof, having Formula I



5 wherein

R^1 and R^2 are independently R^3 or $C(O)R^3$; or

R^1 and R^2 together are $C(R^3)_2$;

10

R^3 is C_8 - C_{24} -alkyl;

X^1 is C_1 - C_6 -alkyl;

15

L^1 is drawn from left to right and is $C(OCH_3)_2$, $NHC(O)$, $C(O)NH$, $OC(O)NH$, $NHC(O)O$, $NHC(O)NH$, $N(N)C(O)$, $C(O)N(N)$, SS , $NHC(O)L^2C(O)O$, $NHC(O)L^2C(O)NH$, $OC(O)L^2C(O)O$, $OC(O)L^2C(O)NH$, $C(O)O$, $OC(O)$, S , O , NH , $CH_2CH(=N)NHR^4C(O)$, $C(=NNHCH_3)R^4$, $C(OCH_3)_2CH_2$, $NHC(O)CH_2$, $C(O)NHCH_2$, $OC(O)NHCH_2$, $NHC(O)OCH_2$, $NHC(O)NHCH_2$, $N(N)C(O)CH_2$, $C(O)N(N)CH_2$, $SSCH_2$, $NHC(O)L^2C(O)OCH_2$, $NHC(O)L^2C(O)NHCH_2$, $OC(O)L^2C(O)OCH_2$, $OC(O)L^2C(O)NHCH_2$, $C(O)OCH_2$, $OC(O)CH_2$, SCH_2 , OCH_2 , $NHCH_2$, $CH_2CH(=N)NHR^4C(O)CH_2$ or $C(=NNHCH_3)R^4CH_2$;

20

R^4 is aryl or heteroaryl;

25

L^2 is C_1 - C_6 -alkyl; and

n is 10-200.

A further embodiment pertains to Cationic-Based Lipid Encapsulation Systems (CaBLES) comprising one or more non-cationic lipids, one or more polyethylene glycol (PEG)-lipid conjugates having Formula I and one or more cationic lipids.

In still a further embodiment, Lipid-Based Particles of the present invention are defined as CaBLES which further comprise one or more therapeutic agent(s). Such Lipid-Based Particles can be used to deliver any of a variety of therapeutic agent(s), preferably said therapeutic agent is a nucleic acid encoded with a product of interest, including but not limited to, RNA, antisense oligonucleotide, a DNA, a plasmid, a ribosomal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA (snRNA), antigens, fragments thereof, proteins, peptides, vaccines and small-molecules or mixtures thereof.

A further embodiment pertains to pharmaceutical compositions comprising a Lipid-Based Particle and a pharmaceutically acceptable carrier.

A further embodiment pertains to a method of treating cancer in a mammal comprising administering thereto a therapeutically acceptable amount of a Lipid-Based Particle. Yet another embodiment pertains to a method of decreasing tumor volume in a mammal comprising administering thereto a therapeutically acceptable amount of a Lipid-Based Particle.

A further embodiment pertains to a method of making CaBLES or Lipid-Based Particles, comprising: (a) mixing the cationic lipid(s), the non-cationic lipid(s) and the PEG-lipid conjugate(s); (b) adding the mixture of step (a) to one or more therapeutic agents; and (c) separating and purifying resulting suspension of step (b).

25

DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates in vivo activity of Lipid-Based Particle 1 (LP1) and Lipid-Based Particle 2 (LP2) versus a non-targeted composition (NTC).

DETAILED DESCRIPTION OF THE INVENTION

This invention pertains to in vitro and in vivo delivery of therapeutic agents. In particular, the invention pertains to compositions that allow for delivery of nucleic acids, including but not limited to RNA, antisense oligonucleotide, a DNA, a plasmid, a
5 ribosomal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA(snRNA), antigens, fragments thereof, proteins, peptides, and small molecules.

Variable moieties of compounds herein are represented by identifiers (capital letters with numerical and/or alphabetical superscripts) and may be specifically
10 embodied.

It is also meant to be understood that a specific embodiment of a variable moiety may be the same or different as another specific embodiment having the same identifier and that asymmetric divalent moieties are drawn from left to right.

As used in the specification and the appended claims, unless specified to the
15 contrary, the following terms have the meaning indicated:

The term “alkenyl,” as used herein, means monovalent, straight or branched chain hydrocarbon moieties having one or more than one carbon-carbon double bonds, such as C₂-alkenyl, C₃-alkenyl, C₄-alkenyl, C₅-alkenyl, C₆-alkenyl and the like.

The term “C₁-C₆-alkylene,” as used herein, means divalent, saturated, straight or
20 branched chain hydrocarbon moieties bonds, such as C₁-alkylene, C₂-alkylene, C₃-alkylene, C₄-alkylene, C₅-alkylene, and C₆-alkylene.

The terms “alkyl,” as used herein, means monovalent, straight or branched chain hydrocarbon moieties such as C₁-alkyl, C₂-alkyl, C₃-alkyl, C₄-alkyl, C₅-alkyl, C₆-alkyl and the like.

25 The term “alkynyl,” as used herein, means monovalent, straight or branched chain hydrocarbon moieties having one or more than one carbon-carbon triple bonds, such as C₂-alkynyl, C₃-alkynyl, C₄-alkynyl, C₅-alkynyl, C₆-alkynyl and the like.

The term “C₁-C₆-alkyl” as used herein, means C₁-alkyl, C₂-alkyl, C₃-alkyl, C₄-alkyl, C₅-alkyl, and C₆-alkyl.

30 The term “C₈-C₂₄-alkenyl,” as used herein, means C₈-alkenyl, C₉-alkenyl, C₁₀-alkenyl, C₁₁-alkenyl, C₁₂-alkenyl, C₁₃-alkenyl, C₁₄-alkenyl, C₁₅-alkenyl, C₁₆-alkenyl, C₁₇-

alkenyl, C₁₈-alkenyl, C₁₉-alkenyl, C₂₀-alkenyl, C₂₁-alkenyl, C₂₂-alkenyl, C₂₃-alkenyl, and C₂₄-alkenyl.

The term "C₈-C₂₄-alkyl," as used herein, means C₈-alkyl, C₉-alkyl, C₁₀-alkyl, C₁₁-alkyl, C₁₂-alkyl, C₁₃-alkyl, C₁₄-alkyl, C₁₅-alkyl, C₁₆-alkyl, C₁₇-alkyl, C₁₈-alkyl, C₁₉-alkyl, C₂₀-alkyl, C₂₁-alkyl, C₂₂-alkyl, C₂₃-alkyl, and C₂₄-alkyl.

The term "aryl," as used herein, means phenyl, a bicyclic aryl or a tricyclic aryl. The bicyclic aryl is naphthyl, a phenyl fused to a cycloalkyl, or a phenyl fused to a cycloalkenyl. The bicyclic aryl is attached to the parent molecular moiety through any carbon atom contained within the bicyclic aryl. Representative examples of the bicyclic aryl include, but are not limited to, dihydroindenyl, indenyl, naphthyl, dihydronaphthalenyl, and tetrahydronaphthalenyl. The tricyclic aryl is anthracene or phenanthrene, or a bicyclic aryl fused to a cycloalkyl, or a bicyclic aryl fused to a cycloalkenyl, or a bicyclic aryl fused to a phenyl. The tricyclic aryl is attached to the parent molecular moiety through any carbon atom contained within the tricyclic aryl. Representative examples of tricyclic aryl ring include, but are not limited to, azulenyl, dihydroanthracenyl, fluorenyl, and tetrahydrophenanthrenyl.

The term "cycloalkane," as used herein, means saturated cyclic or bicyclic hydrocarbon moieties, such as C₃-cycloalkane, C₄-cycloalkane, C₅-cycloalkane, C₆-cycloalkane and the like.

The term "cycloalkyl," as used herein, means monovalent, saturated cyclic and bicyclic hydrocarbon moieties, such as C₃-cycloalkyl, C₄-cycloalkyl, C₅-cycloalkyl, C₆-cycloalkyl and the like.

The term "cycloalkene," as used herein, means cyclic and bicyclic hydrocarbon moieties having one or more than one carbon-carbon double bonds, such as C₅-cycloalkene, C₆-cycloalkene and the like.

The term "cycloalkenyl," as used herein, means monovalent, cyclic hydrocarbon moieties having one or more than one carbon-carbon double bonds, such as C₄-cycloalkenyl, C₅-cycloalkenyl, C₆-cycloalkenyl and the like.

The term "heteroarene," as used herein, means a five-membered or six-membered aromatic ring having at least one carbon atom and one or more than one independently selected nitrogen, oxygen or sulfur atom. The heteroarenes of this invention are

connected through any adjacent atoms in the ring, provided that proper valences are maintained. Examples of heteroarenes include, but are not limited to furan, imidazole, isothiazole, isoxazole, oxadiazole, oxazole, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, thiazole, thiadiazole thiophene, tetrazine, tetrazole, triazine, triazole
5 and the like.

The term "heteroaryl," as used herein, means a monocyclic heteroaryl or a bicyclic heteroaryl. The monocyclic heteroaryl is a 5 or 6 membered ring. The 5 membered ring contains two double bonds and one, two, three or four nitrogen atoms and optionally one oxygen or sulfur atom. The 6 membered ring contains three double bonds
10 and one, two, three or four nitrogen atoms. The 5 or 6 membered heteroaryl is connected to the parent molecular moiety through any carbon atom or any substitutable nitrogen atom contained within the heteroaryl, provided that proper valance is maintained. Representative examples of monocyclic heteroaryl include, but are not limited to, furyl, imidazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, oxazolyl, pyridinyl, pyridazinyl,
15 pyrimidinyl, pyrazinyl, pyrazolyl, pyrrolyl, tetrazolyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, and triazinyl. The bicyclic heteroaryl consists of a monocyclic heteroaryl fused to a phenyl, or a monocyclic heteroaryl fused to a cycloalkyl, or a monocyclic heteroaryl fused to a cycloalkenyl, or a monocyclic heteroaryl fused to a monocyclic heteroaryl. The bicyclic heteroaryl is connected to the parent molecular moiety through any carbon
20 atom or any substitutable nitrogen atom contained within the bicyclic heteroaryl, provided that proper valance is maintained. Representative examples of bicyclic heteroaryl include, but are not limited to, benzofuranyl, benzoxadiazolyl, benzoisoxazole, benzoisothiazole, benzooxazole, 1,3-benzothiazolyl, benzothiophenyl, cinnolinyl, furopyridine, indolyl, indazolyl, isobenzofuran, isoindolyl, isoquinolinyl, naphthyridinyl,
25 oxazolopyridine, quinolinyl, quinoxalinyl and thienopyridinyl.

The term "heterocycloalkane," as used herein, means cycloalkane having one or two or three CH₂ moieties replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties unreplaced or replaced with N and also means cycloalkane having one or two or three CH₂ moieties unreplaced or replaced with independently
30 selected O, S, S(O), SO₂ or NH and one or two CH moieties replaced with N.

The term "heterocycloalkene," as used herein, means cycloalkene having one or two or three CH₂ moieties replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties unreplaced or replaced with N and also means cycloalkene having one or two or three CH₂ moieties unreplaced or replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties replaced with N.

The term "heterocycloalkyl," as used herein, means cycloalkyl having one or two or three CH₂ moieties replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties unreplaced or replaced with N and also means cycloalkyl having one or two or three CH₂ moieties unreplaced or replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties replaced with N.

The term "heterocycloalkenyl," as used herein, means cycloalkenyl having one or two or three CH₂ moieties replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties unreplaced or replaced with N and also means cycloalkenyl having one or two or three CH₂ moieties unreplaced or replaced with independently selected O, S, S(O), SO₂ or NH and one or two CH moieties replaced with N.

The term "cyclic moiety," as used herein, means benzene, cycloalkane, cycloalkyl, cycloalkene, cycloalkenyl, heteroarene, heteroaryl, heterocycloalkane, heterocycloalkyl, heterocycloalkene, heterocycloalkenyl and phenyl.

The term "DSPC," as used herein, means 1,2-distearoyl-*sn*-glycero-3-phosphocholine.

The term, "Chol," as used herein, means cholesterol.

The term, "PEG-Chol," as used herein, means poly(oxy-1,2-ethanediyl)-2000- α -(3 β)-cholest-5-en-3-yl-omega-hydroxy.

The term, "Pal-PEG-Cera," as used herein, means N-palmitoyl-sphingosine-1-[succinyl(methoxypolyethylene glycol)-2000].

The term, "PEG-DMPE," as used herein, means N-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine.

The term, "PEG-DPPE," as used herein, means N-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine.

The term, "PEG-DSPE," as used herein, means N-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.

The term, "PEG-DMG," as used herein, means 1,2-dimyristoyl-sn-glycerol-methoxypolyethyleneglycol-2000.

The term, "PEG-DPG," as used herein, means 1,2-dipalmitoyl-sn-glycerol-methoxypolyethyleneglycol-2000.

5 The term, "PEG-DSG," as used herein, means 1,2-distearoyl-sn-glycerol-methoxypolyethyleneglycol-2000.

The term "MALDI," as used herein, means matrix assisted laser desorption ionization.

10 The term, "particle," as used herein, means a small object that behaves as a whole unit in terms of its transport and properties.

The term, "nanoparticle," as used herein, means any particle having a diameter of less than 1000 nanometers. In some embodiments, nanoparticles have a diameter of 500 or less. In some embodiments, nanoparticles have a diameter of 200 or less.

15 The term "nucleic acid" or "polynucleotide" refers to a polymer containing at least two deoxyribonucleotides or ribonucleotides in either single- or double-stranded form. Nucleic acids include 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
20 such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Unless specifically limited, the terms encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring
25 nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be
30 achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985);

and Cassol et al. (1992); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

"Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups.

Nucleotides include chemically modified nucleotides as described in, e.g., WO 03/74654.

5 "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides. DNA may be in the form of antisense,
10 plasmid DNA, parts of a plasmid DNA, pre-condensed DNA, product of a polymerase chain reaction (PCR), vectors (P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, chromosomal DNA, or derivatives of these groups. The term nucleic acid is used interchangeably with gene, plasmid, cDNA, mRNA, and an interfering RNA molecule (e.g. a synthesized siRNA or an siRNA expressed from a
15 plasmid).

The term, "siRNA," as used herein, means a small inhibitory RNA, and molecules having endogenous RNA bases or chemically modified nucleotides. The modifications shall not abolish cellular activity, but rather impart increased stability and/or increased cellular potency. Examples of chemical modifications include phosphorothioate groups,
20 2'-deoxynucleotide, 2'-OCH₃-containing ribonucleotides, 2'-F-ribonucleotides, 2'-methoxyethyl ribonucleotides or a combination thereof.

The term, "SPC," as used herein, means soybean phosphatidylcholine.

The term "small molecule," as used herein, means antibiotics, antineoplastics, antiinflammatories, antivirals, immunomodulators and agents that act upon the
25 respiratory system, the cardiovascular system, the central nervous system or a metabolic pathway involved with dyslipidemia, diabetes or Syndrome X.

The term, "NTC," as used herein, means a non-targeted composition containing one or more (PEG)-lipid conjugates, one or more non-cationic lipids, one or more cationic lipids, and one or more non-targeted agents such as a non-targeted siRNA (sequence: UGGUUACAUGUUGUGUGA SEQ ID NO: 1).

Compounds

Compounds of this invention may contain asymmetrically substituted carbon atoms in the R or S configuration, wherein the terms "R" and "S" are as defined in Pure Appl. Chem. (1976) 45, 13-10. Compounds having asymmetrically substituted carbon atoms with equal amounts of R and S configurations are racemic at those atoms. Atoms
5 having excess of one configuration over the other are assigned the configuration in excess, preferably an excess of about 85%-90%, more preferably an excess of about 95%-99%, and still more preferably an excess greater than about 99%. Accordingly, this invention is meant to embrace racemic mixtures and relative and absolute diastereoisomers and the compounds thereof.

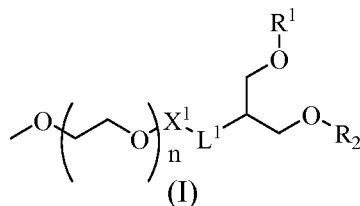
10 Compounds of this invention may also contain carbon-carbon double bonds or carbon-nitrogen double bonds in the E or Z configuration, wherein the term "E" represents higher order substituents on opposite sides of the carbon-carbon or carbon-nitrogen double bond and the term "Z" represents higher order substituents on the same side of the carbon-carbon or carbon-nitrogen double bond as determined by the
15 Cahn-Ingold-Prelog Priority Rules. The compounds of this invention may also exist as a mixture of "E" and "Z" isomers.

Compounds of this invention can exist in an isotopic form containing one or more atoms having an atomic mass or mass number different from the atomic mass or mass number most abundantly found in nature. Isotopes of atoms such as hydrogen, carbon,
20 phosphorous, sulfur fluorine, chlorine, and iodine include, but are not limited to, ^2H , ^3H , ^{14}C , ^{32}P , ^{35}S , ^{18}F , ^{36}Cl , and ^{125}I , respectively. Compounds that contain other isotopes of these and/or other atoms are within the scope of this invention. Compounds containing tritium (^3H) and ^{14}C radioisotopes are preferred in general for their ease in preparation and detectability for radiolabeled compounds. Isotopically labeled compounds of this
25 invention can be prepared by the general methods well known to persons having ordinary skill in the art. Such isotopically labeled compounds can be conveniently prepared by carrying out the procedures disclosed in the Examples and Schemes herein by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

30 Suitable groups for X^1 , L^1 , R^1 , R^2 , R^3 , R^4 , L^2 , and n in compounds of Formula (I) are independently selected. The described embodiments of the present invention may be

combined. Such combination is contemplated and within the scope of the present invention. For example, it is contemplated that embodiments for any of X^1 , L^1 , R^1 , R^2 , R^3 , R^4 , L^2 , and n can be combined with embodiments defined for any other of X^1 , L^1 , R^1 , R^2 , R^3 , R^4 , L^2 , and n .

- 5 One embodiment of this invention pertains to polyethylene glycol (PEG)-lipid conjugates, or mixtures thereof, having Formula I



wherein

- 10 R^1 and R^2 are independently R^3 or $C(O)R^3$; or

R^1 and R^2 together are $C(R^3)_2$;

R^3 is C_8 - C_{24} -alkyl;

15

X^1 is C_1 - C_6 -alkyl;

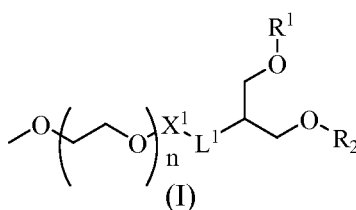
- L^1 is drawn from left to right and is $C(OCH_3)_2$, $NHC(O)$, $C(O)NH$, $OC(O)NH$, $NHC(O)O$, $NHC(O)NH$, $N(N)C(O)$, $C(O)N(N)$, SS , $NHC(O)L^2C(O)O$, $NHC(O)L^2C(O)NH$, $OC(O)L^2C(O)O$, $OC(O)L^2C(O)NH$, $C(O)O$, $OC(O)$, S , O , NH , $CH_2CH(=N)NHR^4C(O)$, $C(=NNHCH_3)R^4$, $C(OCH_3)_2CH_2$, $NHC(O)CH_2$, $C(O)NHCH_2$, $OC(O)NHCH_2$, $NHC(O)OCH_2$, $NHC(O)NHCH_2$, $N(N)C(O)CH_2$, $C(O)N(N)CH_2$, $SSCH_2$, $NHC(O)L^2C(O)OCH_2$, $NHC(O)L^2C(O)NHCH_2$, $OC(O)L^2C(O)OCH_2$, $OC(O)L^2C(O)NHCH_2$, $C(O)OCH_2$, $OC(O)CH_2$, SCH_2 , OCH_2 , $NHCH_2$, $CH_2CH(=N)NHR^4C(O)CH_2$ or $C(=NNHCH_3)R^4CH_2$;
- 20
- 25

R^4 is aryl or heteroaryl;

L^2 is C_1 - C_6 -alkyl; and

5 n is 10-200.

Another embodiment of this invention pertains to polyethylene glycol (PEG)-lipid conjugates, or mixtures thereof, having Formula I



10 wherein

R^1 and R^2 are independently R^3 or $C(O)R^3$;

R^3 is C_8 - C_{24} -alkyl;

15

X^1 is C_1 - C_6 -alkyl;

L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$; and

20 n is 10-200.

In one embodiment of Formula I, R^1 and R^2 are R^3 . In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$. In another embodiment of Formula I, one of R^1 and R^2 is R^3 , and the other is $C(O)R^3$. In another embodiment of Formula I, R^1 and R^2 together are $C(R^3)_2$.

25

In one embodiment of Formula I, R^3 is C_{13} - C_{18} -alkyl. In another embodiment of Formula I, R^3 is C_{13} -alkyl. In another embodiment of Formula I, R^3 is C_{14} -alkyl. In another embodiment of Formula I, R^3 is C_{15} -alkyl. In another embodiment of Formula I, R^3 is C_{16} -alkyl. In another embodiment of Formula I, R^3 is C_{17} -alkyl. In another embodiment of Formula I, R^3 is C_{18} -alkyl. In another embodiment of Formula I, R^3 is tridecanyl-alkyl. In another embodiment of Formula I, R^3 is tetradecanyl-alkyl. In another embodiment of Formula I, R^3 is pentadecanyl-alkyl. In another embodiment of Formula I, R^3 is hexadecanyl-alkyl. In another embodiment of Formula I, R^3 is heptadecanyl-alkyl. In another embodiment of Formula I, R^3 is octadecanyl-alkyl.

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In one embodiment of Formula I, X^1 is C_1 - C_2 -alkyl. In another embodiment of Formula I, X^1 is C_1 -alkyl. In another embodiment of Formula I, X^1 is C_2 -alkyl.

In one embodiment of Formula I, L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$. In another embodiment of Formula I, L^1 is drawn from left to right and is $C(O)NH$. In another embodiment of Formula I, L^1 is drawn from left to right and is $NHC(O)O$.

In one embodiment of Formula I, n is 25-65. In another embodiment of Formula I, n is 35-55. In another embodiment of Formula I, n is 45.

In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$, and n is 10-200. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$, R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$, and n is 10-200. In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $NHC(O)O$, and n is 10-200. In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ and n is 10-200. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$, R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $NHC(O)O$, and n is 10-200. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$,

30

R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ and n is 10-200.

In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$, and n is 45. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$, R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ or $NHC(O)O$, and n is 45. In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $NHC(O)O$, and n is 45. In one embodiment of Formula I, R^1 and R^2 are R^3 , R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ and n is 45. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$, R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $NHC(O)O$, and n is 45. In another embodiment of Formula I, R^1 and R^2 are $C(O)R^3$, R^3 is C_{13} - C_{18} -alkyl, X^1 is C_1 - C_2 -alkyl, L^1 is drawn from left to right and is $C(O)NH$ and n is 45.

Still another embodiment pertains to compounds of Formula I which are
 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 2-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
 hexatetracontaoxaotatriacontahectanamidopropane-1,3-diyl ditetradecanoate,

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaaoctatriacontahectanamidopropane-1,3-diyl dipalmitate,

5 2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaaoctatriacontahectanamidopropane-1,3-diyl distearate,

N-(2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl)-

10 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-

hexatetracontaaoxanonatriacontahectan-139-amide

N-(2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl)-

15 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-

hexatetracontaaoxanonatriacontahectan-139-amide, and

N-(2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl)-

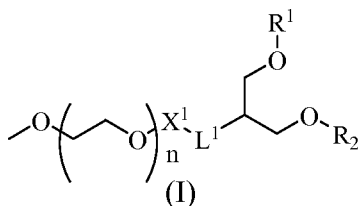
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-

20 hexatetracontaaoxanonatriacontahectan-139-amide.

(PEG)-lipid conjugate-Based Lipid Encapsulation Systems, and Lipid-Based Particles

A still further embodiment pertains to Cationic-Based Lipid Encapsulation Systems (CaBLES) comprising non-cationic lipid(s), polyethylene glycol (PEG)-lipid conjugate(s) having Formula I and cationic lipid(s).

25 A still further embodiment pertains to Cationic-Based Lipid Encapsulation Systems (CaBLES) comprising one or more (PEG)-lipid conjugates having Formula (I)



wherein

R^1 and R^2 are independently R^3 or $C(O)R^3$; or

R^1 and R^2 together are $C(R^3)_2$;

5

R^3 is C_8 - C_{24} -alkyl;

X^1 is C_1 - C_6 -alkyl;

10

L^1 is drawn from left to right and is $C(OCH_3)_2$, $NHC(O)$, $C(O)NH$, $OC(O)NH$, $NHC(O)O$, $NHC(O)NH$, $N(N)C(O)$, $C(O)N(N)$, SS , $NHC(O)L^2C(O)O$, $NHC(O)L^2C(O)NH$, $OC(O)L^2C(O)O$, $OC(O)L^2C(O)NH$, $C(O)O$, $OC(O)$, S , O , NH , $CH_2CH(=N)NHR^4C(O)$, $C(=NNHCH_3)R^4$, $C(OCH_3)_2CH_2$, $NHC(O)CH_2$, $C(O)NHCH_2$, $OC(O)NHCH_2$, $NHC(O)OCH_2$, $NHC(O)NHCH_2$, $N(N)C(O)CH_2$, $C(O)N(N)CH_2$, $SSCH_2$, $NHC(O)L^2C(O)OCH_2$, $NHC(O)L^2C(O)NHCH_2$, $OC(O)L^2C(O)OCH_2$, $OC(O)L^2C(O)NHCH_2$, $C(O)OCH_2$, $OC(O)CH_2$, SCH_2 , OCH_2 , $NHCH_2$, $CH_2CH(=N)NHR^4C(O)CH_2$ or $C(=NNHCH_3)R^4CH_2$;

15

R^4 is aryl or heteroaryl;

20

L^2 is C_1 - C_6 -alkyl; and

n is 10-200; and

25

one or more non-cationic lipids, and one or more cationic lipids.

In still a further embodiment, Lipid-Based Particles of the present invention are defined as CaBLES which further comprise one or more therapeutic agent(s). Therapeutic agents that can be delivered with CaBLES include RNA, antisense oligonucleotide, a

DNA, a plasmid, a ribosomal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA (snRNA), chimeric nucleic acids, an antigen, fragments thereof, a protein, a peptide, small-molecules, or mixtures thereof. This invention describes delivery of RNA's such as small inhibitory RNA or
5 microRNA. The nucleic acid can have varying lengths (10-200 bps) and structures (hairpins, single/double strands, bulges, nicks/gaps, mismatches) and processed in the cell to provide active gene silencing. In certain embodiments of this invention, a double-stranded siRNA (dsRNA) can have the same number of nucleotides on each strand (blunt ends) or asymmetric ends (overhangs). The overhang of 1-2 nucleotides can be present on
10 the sense and/or the antisense strand, as well as present on the 5'- and/ or the 3'-ends of a given strand.

In one embodiment, the therapeutic agent is RNA, antisense oligonucleotide, a DNA, a plasmid, a ribozymal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA (snRNA), an antigen,
15 fragments thereof, a protein, a peptide, a small-molecule, or a mixture thereof.

In certain embodiments, the PEG lipid conjugate of the Lipid-Based Particle can have a ligand attached, such as a targeting ligand or a chelating moiety. Suitable targeting ligands include, but are not limited to, a compound or device with a reactive functional group and include lipids, amphipathic lipids, carrier compounds, bioaffinity
20 compounds, biomaterials, biopolymers, biomedical devices, analytically detectable compounds, therapeutically active compounds, enzymes, peptides, proteins, antibodies, immune stimulators, radiolabels, fluorogens, biotin, drugs, haptens, DNA, RNA, polysaccharides, liposomes, virosomes, micelles, immunoglobulins, functional groups, other targeting moieties, or toxins.

In another embodiment, a targeting ligand (moiety) is conjugated to the periphery
25 of the PEG-lipid in a Lipid-Based Particle formulation. Preferably, the targeting moiety is a ligand of a receptor present on a target cell and the receptor is preferentially expressed by the target cell versus a non-target cell. In one aspect, the targeting moiety is an antibody or fragments thereof. In one aspect, the targeting moiety is a small protein, or
30 peptide. In another aspect, the targeting moiety is a small-molecule.

In still a further embodiment, these Lipid-Based Particles are nanoparticles and have mean diameter sizes of about 50-300 nm, of which 50-250 nm is preferred and 50-200 nm is most preferred.

A further embodiment pertains to CaBLES or Lipid-Base Particles wherein the
 5 PEG lipid conjugate(s) are about 0.1-20 weight/weight % of total lipid in particle, the non-cationic lipid(s) are about 1-30 weight/weight % of total lipid in particle, the cholesterol is about 5-45 weight/weight % of total lipid in particle, and the cationic lipid(s) are about 5-60 weight/weight % of total lipid in particle.

A further embodiment pertains to CaBLES or Lipid-Base Particles wherein the
 10 PEG lipid conjugate(s) are about 0.1-20 weight/weight % of total lipid in particle, the DSPC is about 1-30 weight/weight % of total lipid in particle, the cholesterol is about 5-45 weight/weight % of total lipid in particle, and the cationic lipid(s) are about 5-60 weight/weight % of total lipid in particle.

A further embodiment pertains to a pharmaceutical composition comprising a
 15 Lipid-Based Particle and a pharmaceutically acceptable carrier.

A further embodiment pertains to a pharmaceutical composition comprising a Lipid-Based Particle and a pharmaceutically acceptable carrier, wherein the Lipid-Based Particle comprises cholesterol, DSPC, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
 20 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate and one or more nucleic acids.

A further embodiment pertains to a pharmaceutical composition comprising a Lipid-Based Particle and a pharmaceutically acceptable carrier, wherein 2-
 25 (tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, DSPC is about 1-30 weight/weight % of total lipid in particle,
 30 cholesterol is about 5-45 weight/weight % of total lipid in particle, and 1-(2,3-

bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

A further embodiment pertains to a Lipid-Based Particle, wherein the non-cationic lipids are cholesterol and DSPC, the cationic lipid is 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, the PEG-lipid conjugate is 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate, and the therapeutic agent is siRNA.

A further embodiment pertains to a Lipid-Based Particle, wherein 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, DSPC is about 1-30 weight/weight % of total lipid in particle, cholesterol is about 5-45 weight/weight % of total lipid in particle, and 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

A further embodiment pertains to a pharmaceutical composition comprising a Lipid-Based Particle and a pharmaceutically acceptable carrier, wherein the Lipid-Based Particle comprises, cholesterol, DSPC, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate and one or more nucleic acids.

A further embodiment pertains to a pharmaceutical composition comprising a Lipid-Based Particle and a pharmaceutically acceptable carrier, wherein the 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of

total lipid in particle, the DSPC is about 1-30 weight/weight % of total lipid in particle, the cholesterol is about 5-45 weight/weight % of total lipid in particle, and the 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

5 A further embodiment pertains to a Lipid-Based Particle, wherein the non-cationic lipids are cholesterol and DSPC, the cationic lipid is 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, the PEG-lipid conjugate is 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 10 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate, and the therapeutic agent is siRNA.

A further embodiment pertains to a Lipid-Based Particle, wherein the 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 15 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, the DSPC is about 1-30 weight/weight % of total lipid in particle, the cholesterol is about 5-45 weight/weight % of total lipid in particle, and the 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % 20 of total lipid in particle.

A further embodiment pertains to functional CaBLES comprising one or more (PEG)-lipid conjugates of Formula 1, one or more non-cationic lipids, and one or more cationic lipids effectively encapsulate nucleic acids, such as siRNA, with efficiencies from about 50-100%.

A further embodiment pertains to functional CaBLES comprising one or more (PEG)-lipid conjugates of Formula 1, one or more non-cationic lipids, and one or more cationic lipids effectively encapsulate nucleic acids, such as siRNA, with efficiencies from about 80-100%.

A further embodiment pertains to a Lipid-Based Particle, wherein the ratio of one or more (PEG)-lipid conjugates, one or more non-cationic lipids, and one or more

cationic lipids of claim 1, to one or more therapeutic agents is between about 50:1 to about 5:1.

A further embodiment pertains to a Lipid-Based Particle,, wherein the ratio of one or more (PEG)-lipid conjugates, one or more non-cationic lipids, and one or more
 5 cationic lipids of claim 1, to one or more therapeutic agents is between about 30:1 to about 10:1.

A further embodiment pertains to examples of non-cationic lipids that are useful for the practice of this invention which include, but are not limited to, cholesterol, cholesterol sulfate, ceramide, sphingomyelin, lecithin, sphingomyelin, egg
 10 sphingomyelin, milk sphingomyelin; egg phosphatidylcholine, hydrogenated egg phosphatidylcholine, hydrogenated soybean phosphatidylethanolamine, egg phosphatidylethanolamine, hydrogenated soybean phosphatidylcholine, soybean phosphatidylcholine, 1,2-dilauroyl-sn-glycerol, 1,2-dimyristoyl-sn-glycerol, 1,2-dipalmitoyl-sn-glycerol, 1,2-distearoyl-sn-glycerol, 1,2-dilauroyl-sn-glycero-3-
 15 phosphatidic acid, 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid, 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, 1,2-distearoyl-sn-glycero-3-phosphatidic acid, 1,2-diarachidoyl-sn-glycero-3-phosphocholine, 1,2-dilauroyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine, dioleoylphosphatidylcholine, 1,2-dierucoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-
 20 palmitoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine;
 25 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-lyso-sn-glycero-3-phosphocholine, 1-palmitoyl-2-lyso-sn-glycero-3-phosphocholine, 1-stearoyl-2-lyso-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-O-ethyl-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; 1,2-distearoyl-sn-glycero-3-phosphocholine; 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine,
 30 dioleoylphosphatidylethanolamine, palmitoyl-oleoyl-phosphatidylethanolamine, dioleoylphosphatidylglycerol, 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine,

1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilauroyl-sn-glycero-3-phosphoglycerol, 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol, 1,2-dimyristoyl-sn-glycero-3-phospho-sn-1-glycerol, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol, 1,2-distearoyl-sn-glycero-3-phosphoglycerol, 1,2-distearoyl-sn-glycero-3-phospho-sn-1-glycerol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine, 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine, 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine, 1,2-distearoyl-sn-glycero-3-phospho-L-serine, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine, and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine or a mixture thereof.

A further embodiment pertains to examples of cationic lipids that are useful for the practice of this invention which include, but are not limited to, N,N-dioleoyl-N,N-dimethylammonium chloride, DC-Chol; 1,3-dioleoyloxy-2-(6-carboxyspermyl)-propyl amide, dioctadecylamidoglycyl spermine, N,N-distearyl-N,N-dimethylammonium bromide, N-(2,3-dioleoyloxy)propyl)-N,N-dimethylammonium chloride, 1,2-dioleoyl-3-trimethylammonium-propane chloride, 1,2-dilinoeoyl-3-dimethylammonium-propane, N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride, 1,2-dioleoyl-3-dimethylammonium propane, 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane; didodecyltrimethylammonium bromide, dioleoyloxy-N-(2-sperminecarboxamido)ethyl)-N,N-dimethyl-1-propanaminiumtrifluoroacetate, 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl ammonium bromide, 1,2-dioleoylcarbonyl-3-dimethylammoniumpropane, tetramethyltetrapalmitoyl spermine, tetramethyltetraoleyl spermine, tetramethyldioleoyl spermine, tetramethyltetramyristyl spermine, tetramethyltetralauryl spermine, 1-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)pyrrolidine; N,N-dimethyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(3-(1H-imidazol-1-yl)propyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; 1-methyl-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)piperazine; 4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)morpholine; N-(2-

((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N'-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)ethane-1,2-diamine; N-(2-(4-methylpiperazin-1-yl)ethyl)-N-(2-
 5 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(2-(1H-imidazol-4-yl)ethyl)-N-(2-((9Z,12Z)-octadeca-
 9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N,N-dimethyl-N-(3-(4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)piperazin-1-yl)propyl)amine; 1,3-bis((9Z,12Z)-octadeca-9,12-
 10 dienyloxy)propan-2-amine; N-((1-methylpiperidin-4-yl)methyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-
 (2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; N-methyl-N-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N-(3-((4-methylpiperazin-1-
 15 yl)methyl)benzyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-methyl-N-((1-methylpiperidin-4-yl)methyl)-N-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N,N,N'-trimethyl-N'-(2-((9Z,12Z)-octadeca-9,12-
 20 dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)propane-1,3-diamine; N-methyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; 1-(2-(1H-imidazol-1-yl)ethyl)-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)piperazine; N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-
 25 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-((2-pyrrolidin-1-ylpyridin-3-yl)methyl)amine; (9Z,9'Z,12Z,12'Z)-2-(4-methylpiperazin-1-yl)propane-1,3-diyl
 dioctadeca-9,12-dienoate; (9Z,9'Z,12Z,12'Z)-2-(3-(pyrrolidin-1-yl)propylamino)propane-1,3-diyl dioctadeca-9,12-dienoate; 1-methyl-4-(3-((9Z,12Z)-octadeca-9,12-dienyloxy)-2-
 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)piperazine; 1-(3-((9Z,12Z)-octadeca-
 30 9,12-dienyloxy)-2-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)pyrrolidine; N-(3-aminopropyl)-N'-{3-[(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-[(9Z,12Z)-octadeca-

9,12-dienyloxy)methyl}ethyl)amino]propyl}butane-1,4-diamine; N-(3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy)methyl}propyl)-N-(3-
 pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N-(3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-
 {[(9Z,12Z)-octadeca-9,12-dienyloxy)methyl}propyl)amine; 3-[(9Z,12Z)-octadeca-9,12-
 5 dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy)methyl}propyl 2-
 (diethylamino)ethylcarbamate; 3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-
 octadeca-9,12-dienyloxy)methyl}propyl 2-pyrrolidin-1-ylethylcarbamate; 3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy)methyl}propyl 2-
 (dimethylamino)ethylcarbamate; 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-{[(9Z,12Z)-
 10 octadeca-9,12-dienyloxy)methyl}ethyl)-4-(2-pyrrolidin-1-ylethyl)piperazine; N-(2-[(9Z)-
 octadec-9-enyloxy]-1-{[(9Z)-octadec-9-enyloxy)methyl}ethyl)-N-(3-pyrrolidin-1-
 ylpropyl)amine, 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-{[(9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl}ethyl)azetidine, 2-methyl-1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-
 {[(9Z,12Z)-octadeca-9,12-dienyloxy)methyl}ethyl)aziridine, 1-{3,4-bis[(9Z,12Z)-
 15 octadeca-9,12-dienyloxy]butyl}piperidine, 4-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}morpholine, N,N-diethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, N,N-dimethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 phenylpiperazine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 20 methylpiperazine, N-(2-methoxyethyl)-N-methyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-
 methoxyphenyl)piperazine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-
 N,N',N'-trimethylethane-1,2-diamine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}-N-methyl-N-(2-pyridin-2-ylethyl)amine, N-benzyl-N-{3,4-
 25 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-methylamine, N-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}-N-(4-fluorobenzyl)-N-methylamine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-fluorophenyl)piperazine, N-benzyl-N-
 {3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-ethylamine, N-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}-N-ethyl-N',N'-dimethylethane-1,2-diamine, 1-{3,4-
 30 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpiperidin-4-amine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpyrrolidin-3-amine, N,N-

bis(2-methoxyethyl)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butan-1-amine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-methoxypiperidine, 1-[(3R)-3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl]pyrrolidine, 1-[(3S)-3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl]pyrrolidine, N-[(3R)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 5 dienyloxy]butyl]-N,N-diethylamine, N-[(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl]-N,N-diethylamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}pyrrolidine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-N,N-diethylamine, 2-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-1-methylpyrrolidine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 10 dienyloxy]butoxy}ethyl)aziridine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-4-methylpiperazine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-N,N-dimethylamine, 4-(diethylamino)-2-[(9Z,12Z)-octadeca-
 9,12-dienoyloxy]butyl (9Z,12Z)-octadeca-9,12-dienoate, 1-(2-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butoxy}ethyl)pyrrolidine, N,N-diethyl-N-(2-{2-[(8Z,11Z)-
 15 heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-
 yl}ethyl)amine, 1-[[[(9Z)-octadec-9-enoyloxy]methyl]-3-pyrrolidin-1-ylpropyl (9Z)-
 octadec-9-enoate, 1-{3,4-bis[(9Z)-octadec-9-enyloxy]butyl}pyrrolidine, 1-
 {[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoyloxy]methyl}-3-pyrrolidin-1-ylpropyl
 (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate, (3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 20 dienyloxy]butyl 3-pyrrolidin-1-ylpropylcarbamate, 1-[3,4-
 bis(octadecyloxy)butyl]pyrrolidine, 1-[3,4-bis(hexadecyloxy)butyl]pyrrolidine, 1-{3,4-
 bis[(9E)-hexadec-9-enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E)-octadec-9-
 enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E,12E)-octadeca-9,12-
 dienyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9Z,12Z,15Z)-octadeca-9,12,15-
 25 trienyloxy]butyl}pyrrolidine, N¹-[(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl]-N³,N³-diethyl-beta-alaninamide, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}-N-[3-(1H-imidazol-1-yl)propyl]amine, N-{3,4-bis[(9Z,12Z)-octadeca-
 9,12-dienyloxy]butyl}-N,N',N'-trimethylpropane-1,3-diamine, 1-(1-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}pyrrolidin-3-yl)-1H-imidazole, N-{3,4-bis[(9Z,12Z)-
 30 octadeca-9,12-dienyloxy]butyl}-N-(3-pyrrolidin-1-ylpropyl)amine, N-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}-N',N'-dimethylpropane-1,3-diamine, 1-{3,4-

bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}azetidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2-methylpyrrolidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2,5-dimethylpyrrolidine, are 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-1H-imidazole, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methyl-1,4-diazepane, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-phenylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-pyridin-2-ylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperidine, 4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)morpholine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-((2S)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-ethylpiperazine, N-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N-methyl-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine, N-(2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)ethyl)-N,N-dimethylamine, 1-((2S)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-(2-pyrrolidin-1-ylethyl)piperazine, 2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)pyrimidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N,N-diethylpyrrolidin-3-amine, 1-((9Z,12Z)-octadeca-9,12-dienyloxy)-3-pyrrolidin-1-ylpropan-2-ol, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 1-({2-[(8Z,11Z)-heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-yl}methyl)pyrrolidine, 1-{2,3-bis[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]propyl}pyrrolidine, 1-{3-[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9E,12E)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2-[(9E,12E)-octadeca-9,12-dienyloxy]-3-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-[2,3-bis(tetradecyloxy)propyl]pyrrolidine, 1-[2,3-bis(octadecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-[2,3-

bis(dodecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidin-3-ol, 1-{3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}-N,N-dimethylpyrrolidin-3-amine and 1-[3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-(tetradecyloxy)propyl]pyrrolidine.

Cationic lipids are described in, e.g., US App. No. 12/425,198, which was filed on April 16, 2009, and is incorporated herein by reference.

Cationic lipids are described in, e.g., US App. No. 12/425,266, which was filed on April 16, 2009, and is incorporated herein by reference.

Cationic lipids are described in, e.g., US App. No. 12/425,254, which was filed on April 16, 2009, and is incorporated herein by reference.

In still a further embodiment, the cationic lipids of the CaBLES and Lipid-Based Particles comprises about 2 to about 60 weight/weight percent of total lipid in the particle.

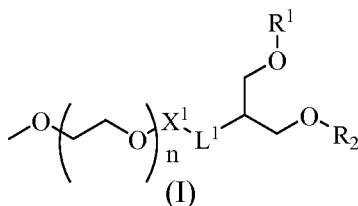
In still a further embodiment, the non-cationic lipids of the Cables and Lipid-Based Particles comprises about 5 to about 90 weight/weight percent of total lipid in the particle.

In still a further embodiment, the PEG-lipid conjugates of the CaBLES and Lipid-Based Particles comprises from 0.1 to about 20 weight/weight percent of total lipid in the particle.

Methods of Treatment and Methods of Making Lipid-Based Particles

Still another embodiment pertains to a method of treating cancer in a mammal comprising administering thereto a Lipid-Based Particle.

Still another embodiment comprises methods of treating cancer in a mammal comprising administering thereto a Lipid-Based Particle comprising one or more polyethylene glycol-lipid conjugates having Formula (I)



wherein

R^1 and R^2 are independently R^3 or $C(O)R^3$; or

R^1 and R^2 together are $C(R^3)_2$;

5

R^3 is C_8 - C_{24} -alkyl;

X^1 is C_1 - C_6 -alkyl;

10

L^1 is drawn from left to right and is $C(OCH_3)_2$, $NHC(O)$, $C(O)NH$, $OC(O)NH$, $NHC(O)O$, $NHC(O)NH$, $N(N)C(O)$, $C(O)N(N)$, SS , $NHC(O)L^2C(O)O$, $NHC(O)L^2C(O)NH$, $OC(O)L^2C(O)O$, $OC(O)L^2C(O)NH$, $C(O)O$, $OC(O)$, S , O , NH , $CH_2CH(=N)NHR^4C(O)$, $C(=NNHCH_3)R^4$, $C(OCH_3)_2CH_2$, $NHC(O)CH_2$, $C(O)NHCH_2$, $OC(O)NHCH_2$, $NHC(O)OCH_2$, $NHC(O)NHCH_2$, $N(N)C(O)CH_2$, $C(O)N(N)CH_2$, $SSCH_2$, $NHC(O)L^2C(O)OCH_2$, $NHC(O)L^2C(O)NHCH_2$, $OC(O)L^2C(O)OCH_2$, $OC(O)L^2C(O)NHCH_2$, $C(O)OCH_2$, $OC(O)CH_2$, SCH_2 , OCH_2 , $NHCH_2$, $CH_2CH(=N)NHR^4C(O)CH_2$ or $C(=NNHCH_3)R^4CH_2$;

15

R^4 is aryl or heteroaryl;

20

L^2 is C_1 - C_6 -alkyl; and

n is 10-200 and

25

one or more non-cationic lipids, one or more cationic lipids, and one or more therapeutic agents.

A further embodiment pertains to a method of making CaBLES or Lipid-Based Particles, comprising: (a) mixing the cationic lipid(s), the non-cationic lipid(s) and the

PEG-lipid conjugate(s); (b) adding the mixture of step (a) to one or more therapeutic agents; and (c) separating and purifying resulting suspension of step (b).

A further embodiment pertains to a method of making Lipid-Based Particles wherein the mixture of step (a) and one or more said therapeutic agents are warmed to
5 about 60° C prior to the addition of the mixture of step (a) to one or more therapeutic agents via needle injection.

Pharmaceutical Compositions and Methods of Administration

Therapeutically effective amounts of Lipid-Based Particles of this invention depend on recipient of treatment, disease treated and severity thereof, composition
10 comprising it, time of administration, route of administration, duration of treatment, potency, rate of clearance and whether or not another drug is co-administered. The amount of Lipid-Based Particles of this invention used to make compositions to be administered daily to a patient in a single dose or in divided doses is from about 0.001 to about 200 mg/kg body weight. Single dose compositions contain these amounts or a
15 combination of submultiples thereof.

One embodiment pertains to a pharmaceutical composition comprising one or more (PEG)-lipid conjugates of Formula 1, one or more non-cationic lipids, one or more cationic lipids, one or more therapeutic agents, and a pharmaceutically acceptable excipient.

Lipid-Based Particles of this invention may be administered, for example, buccally, ophthalmically, orally, osmotically, parenterally (intramuscularly, intraperitoneally intrasternally, intravenously, subcutaneously), rectally, topically, transdermally, vaginally and intraarterially as well as by intraarticular injection, infusion,
20 and placement in the body, such as, for example, the vasculature.

Lipid-Based Particles may be administered with or without an excipient. Excipients include, but are not limited to, encapsulators and additives such as absorption accelerators, antioxidants, binders, buffers, coating agents, coloring agents, diluents, disintegrating agents, emulsifiers, extenders, fillers, flavoring agents, humectants,
25 lubricants, perfumes, preservatives, propellants, releasing agents, sterilizing agents, sweeteners, solubilizers, wetting agents, mixtures thereof and the like.

Excipients for preparation of compositions comprising Lipid-Based Particles to be administered orally include, but are not limited to, agar, alginic acid, aluminum hydroxide, benzyl alcohol, benzyl benzoate, 1,3-butylene glycol, carbomers, castor oil, cellulose, cellulose acetate, cocoa butter, corn starch, corn oil, cottonseed oil, cross-
5 povidone, diglycerides, ethanol, ethyl cellulose, ethyl laureate, ethyl oleate, fatty acid esters, gelatin, germ oil, glucose, glycerol, groundnut oil, hydroxypropylmethyl cellulose, isopropanol, isotonic saline, lactose, magnesium hydroxide, magnesium stearate, malt, mannitol, monoglycerides, olive oil, peanut oil, potassium phosphate salts, potato starch, povidone, propylene glycol, Ringer's solution, safflower oil, sesame oil, sodium
10 carboxymethyl cellulose, sodium phosphate salts, sodium lauryl sulfate, sodium sorbitol, soybean oil, stearic acids, stearyl fumarate, sucrose, surfactants, talc, tragacanth, tetrahydrofurfuryl alcohol, triglycerides, water, mixtures thereof and the like. Excipients for preparation of compositions comprising a compound having formula (I) to be administered ophthalmically or orally include, but are not limited to, 1,3-butylene glycol,
15 castor oil, corn oil, cottonseed oil, ethanol, fatty acid esters of sorbitan, germ oil, groundnut oil, glycerol, isopropanol, olive oil, polyethylene glycols, propylene glycol, sesame oil, water, mixtures thereof and the like. Excipients for preparation of compositions comprising a compound having formula (I) to be administered osmotically include, but are not limited to, chlorofluorohydrocarbons, ethanol, water, mixtures
20 thereof and the like. Excipients for preparation of compositions comprising a compound having formula (I) to be administered parenterally include, but are not limited to, 1,3-butanediol, castor oil, corn oil, cottonseed oil, dextrose, germ oil, groundnut oil, liposomes, oleic acid, olive oil, peanut oil, Ringer's solution, safflower oil, sesame oil, soybean oil, U.S.P. or isotonic sodium chloride solution, water, mixtures thereof and the
25 like. Excipients for preparation of compositions comprising a compound having formula (I) to be administered rectally or vaginally include, but are not limited to, cocoa butter, polyethylene glycol, wax, mixtures thereof and the like.

Combination Therapy

The present invention further provides methods of using a compound,
30 formulation, or composition of the invention in combination with one or more additional active agents.

Lipid-Based Particles are expected to be useful when used with: alkylating agents, angiogenesis inhibitors, antibodies, antimetabolites, antimitotics, antiproliferatives, aurora kinase inhibitors, apoptosis promoters (for example, Bcl-xL, Bcl-w and Bfl-1) inhibitors, Bcr-Abl kinase inhibitors, BiTE (Bi-Specific T cell Engager) antibodies, biologic response modifiers, cyclin-dependent kinase inhibitors, cell cycle inhibitors, cyclooxygenase-2 inhibitors, DVD's, leukemia viral oncogene homolog (ErbB2) receptor inhibitors, growth factor inhibitors, heat shock protein (HSP)-90 inhibitors, histone deacetylase (HDAC) inhibitors, hormonal therapies, immunologicals, inhibitors of apoptosis proteins (IAP's) intercalating antibiotics, kinase inhibitors, mammalian target of rapamycin inhibitors, microRNA's mitogen-activated extracellular signal-regulated kinase inhibitors, multivalent binding proteins, non-steroidal anti-inflammatory drugs (NSAIDs), poly ADP (adenosine diphosphate)-ribose polymerase (PARP) inhibitors, platinum chemotherapeutics, polo-like kinase (Plk) inhibitors, proteasome inhibitors, purine analogs, pyrimidine analogs, receptor tyrosine kinase inhibitors, retinoids/deltoids plant alkaloids, small inhibitory ribonucleic acids (siRNA's), topoisomerase inhibitors, combinations thereof and the like.

The pharmaceutical composition and the method of the present invention may further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of the above-mentioned pathological conditions.

A BiTE antibody is a bi-specific antibody that directs T-cells to attach cancer cells by simultaneously binding the two cells. The T-cell then attacks the target cancer cell. Exemplary BiTE antibodies include adecatumumab (Micromet MT201), blinatumomab (Micromet MT103) and the like.

SiRNA's are molecules having endogenous RNA bases or chemically modified nucleotides. The modifications shall not abolish cellular activity, but rather impart increased stability and/or increased cellular potency. Examples of chemical modifications include phosphorothioate groups, 2'-deoxynucleotide, 2'-OCH₃-containing ribonucleotides, 2'-F-ribonucleotides, 2'-methoxyethyl ribonucleotides or a combination thereof. The siRNA can have varying lengths (10-200 bps) and structures (hairpins, single/double strands, bulges, nicks/gaps, mismatches) and processed in the cell to provide active gene silencing. In certain embodiments, a double-stranded siRNA

(dsRNA) can have the same number of nucleotides on each strand (blunt ends) or asymmetric ends (overhangs). The overhang of 1-2 nucleotides can be present on the sense and/or the antisense strand, as well as present on the 5'- and/ or the 3'-ends of a given strand.

5 Multivalent binding proteins are binding proteins comprising two or more antigen binding sites. The multivalent binding protein is preferably engineered to have the three or more antigen binding sites and is generally not a naturally occurring antibody. The term "multispecific binding protein" means a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins are
10 tetravalent or multivalent binding proteins comprising two or more antigen binding sites. Such DVDs may be monospecific, i.e., capable of binding one antigen or multispecific, i.e., capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as DVD Ig. Each half of a DVD Ig comprises a heavy chain
15 DVD polypeptide, a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site.

Alkylating agents include altretamine, AMD-473, AP-5280, apaziquone, bendamustine, brostallicin, busulfan, carboquone, carmustine (BCNU), chlorambucil,
20 CLORETAZINE[®] (laromustine, VNP 40101M), cyclophosphamide, decarbazine, estramustine, fotemustine, glufosfamide, ifosfamide, KW-2170, lomustine (CCNU), mafosfamide, melphalan, mitobronitol, mitolactol, nimustine, nitrogen mustard N-oxide, ranimustine, temozolomide, thiotepa, TREANDA[®] (bendamustine), treosulfan, rofosfamide and the like.

25 Angiogenesis inhibitors include endothelial-specific receptor tyrosine kinase (Tie-2) inhibitors, epidermal growth factor receptor (EGFR) inhibitors, insulin growth factor-2 receptor (IGFR-2) inhibitors, matrix metalloproteinase-2 (MMP-2) inhibitors, matrix metalloproteinase-9 (MMP-9) inhibitors, platelet-derived growth factor receptor (PDGFR) inhibitors, thrombospondin analogs, vascular endothelial growth factor
30 receptor tyrosine kinase (VEGFR) inhibitors and the like.

Antimetabolites include ALIMTA[®] (metrexed disodium, LY231514, MTA), 5-azacitidine, XELODA[®] (capecitabine), carmofur, LEUSTAT[®] (cladribine), clofarabine, cytarabine, cytarabine ocfosfate, cytosine arabinoside, decitabine, deferoxamine, doxifluridine, eflornithine, EICAR (5-ethynyl-1-β -D-ribofuranosylimidazole-4-carboxamide), enocitabine, ethynylcytidine, fludarabine, 5-fluorouracil alone or in combination with leucovorin, GEMZAR[®] (gemcitabine), hydroxyurea, ALKERAN[®] (melphalan), mercaptopurine, 6-mercaptopurine riboside, methotrexate, mycophenolic acid, nelarabine, nolatrexed, ocfosfate, pelitrexol, pentostatin, raltitrexed, Ribavirin, triapine, trimetrexate, S-1, tiazofurin, tegafur, TS-1, vidarabine, UFT and the like.

Bcl-2 proteins inhibitors include AT-101 ((-)-gossypol), GENASENSE[®] (G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide)), IPI-194, IPI-565, N-(4-(4-((4'-chloro(1,1'-biphenyl)-2-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide (ABT-737), N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (ABT-263), GX-070 (obatoclox) and the like.

Bcr-Abl kinase inhibitors include DASATINIB[®] (BMS-354825), GLEEVEC[®] (imatinib) and the like.

CDK inhibitors include AZD-5438, BMI-1040, BMS-032, BMS-387, CVT-2584, flavopyridol, GPC-286199, MCS-5A, PD0332991, PHA-690509, seliciclib (CYC-202, R-roscovitine), ZK-304709 and the like.

COX-2 inhibitors include ABT-963, ARCOXIA[®] (etoricoxib), BEXTRA[®] (valdecoxib), BMS347070, CELEBREX[®] (celecoxib), COX-189 (lumiracoxib), CT-3, DERAMAXX[®] (deracoxib), JTE-522, 4-methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoylphenyl)-1H-pyrrole, MK-663 (etoricoxib), NS-398, parecoxib, RS-57067, SC-58125, SD-8381, SVT-2016, S-2474, T-614, VIOXX[®] (rofecoxib) and the like.

EGFR inhibitors include ABX-EGF, anti-EGFR immunoliposomes, EGF-vaccine, EMD-7200, ERBITUX[®] (cetuximab), HR3, IgA antibodies, IRESSA[®] (gefitinib), TARCEVA[®] (erlotinib or OSI-774), TP-38, EGFR fusion protein, TYKERB[®] (lapatinib) and the like.

5 ErbB2 receptor inhibitors include CP-724-714, CI-1033 (canertinib), HERCEPTIN[®] (trastuzumab), TYKERB[®] (lapatinib), OMNITARG[®] (2C4, pertuzumab), TAK-165, GW-572016 (ionafarnib), GW-282974, EKB-569, PI-166, dHER2 (HER2 vaccine), APC-8024 (HER-2 vaccine), anti-HER/2neu bispecific antibody, B7.her2IgG3, AS HER2 trifunctional bispecific antibodies, mAB AR-209, mAB 2B-1 and the like.

10 Histone deacetylase inhibitors include depsipeptide, LAQ-824, MS-275, trapoxin, suberoylanilide hydroxamic acid (SAHA), TSA, valproic acid and the like.

HSP-90 inhibitors include 17-AAG-nab, 17-AAG, CNF-101, CNF-1010, CNF-2024, 17-DMAG, geldanamycin, IPI-504, KOS-953, MYCOGRAB[®] (human recombinant antibody to HSP-90), NCS-683664, PU24FcI, PU-3, radicicol, SNX-2112, 15 STA-9090 VER49009 and the like.

Inhibitors of apoptosis proteins include ApoMab (a fully human affinity-matured IgG1 monoclonal antibody), antibodies that target TRAIL or death receptors (e.g., pro-apoptotic receptor agonists DR4 and DR5), conatumumab, ETR2-ST01, GDC0145, (lexatumumab), HGS-1029, LBY-135, PRO-1762 and tratuzumab.

20 MEK inhibitors include ARRY-142886, ARRY-438162 PD-325901, PD-98059 and the like.

mTOR inhibitors include AP-23573, CCI-779, everolimus, RAD-001, rapamycin, temsirolimus and the like.

Non-steroidal anti-inflammatory drugs include AMIGESIC[®] (salsalate), 25 DOLOBID[®] (diflunisal), MOTRIN[®] (ibuprofen), ORUDIS[®] (ketoprofen), RELAFEN[®] (nabumetone), FELDENE[®] (piroxicam), ibuprofen cream, ALEVE[®] (naproxen) and NAPROSYN[®] (naproxen), VOLTAREN[®] (diclofenac), INDOCIN[®] (indomethacin), CLINORIL[®] (sulindac), TOLECTIN[®] (tolmetin), LODINE[®] (etodolac), TORADOL[®] (ketorolac), DAYPRO[®] (oxaprozin) and the like.

30 PDGFR inhibitors include C-451, CP-673, CP-868596 and the like.

Platinum chemotherapeutics include cisplatin, ELOXATIN[®] (oxaliplatin) eptaplatin, lobaplatin, nedaplatin, PARAPLATIN[®] (carboplatin), satraplatin and the like.

Polo-like kinase inhibitors include BI-2536 and the like.

Thrombospondin analogs include ABT-510, ABT-567, TSP-1 and the like.

- 5 VEGFR inhibitors include AVASTIN[®] (bevacizumab), ABT-869, AEE-788, ANGIOZYME[™] (a ribozyme that inhibits angiogenesis (Ribozyme Pharmaceuticals (Boulder, CO.) and Chiron, (Emeryville, CA)), axitinib (AG-13736), AZD-2171, CP-547,632, IM-862, MACUGEN (pegaptamib), NEXAVAR[®] (sorafenib, BAY43-9006), pazopanib (GW-786034), vatalanib (PTK-787, ZK-222584), SUTENT[®]
10 (sunitinib, SU-11248), VEGF trap, ZACTIMA[™] (vandetanib, ZD-6474) and the like.

- Antibiotics include intercalating antibiotics aclarubicin, actinomycin D, amrubicin, annamycin, adriamycin, BLENOXANE[®] (bleomycin), daunorubicin, CAELYX[®] or MYOCET[®] (liposomal doxorubicin), elsamitrucin, epirubicin, glarbuicin, ZAVEDOS[®] (idarubicin), mitomycin C, nemorubicin, neocarzinostatin, peplomycin,
15 pirarubicin, rebeccamycin, stimalamer, streptozocin, VALSTAR[®] (valrubicin), zinostatin and the like.

- Topoisomerase inhibitors include aclarubicin, 9-aminocamptothecin, amonafide, amsacrine, becatecarin, belotecan, BN-80915, CAMPTOSAR[®] (irinotecan hydrochloride), camptothecin, CARDIOXANE[®] (dexrazoxine), diflomotecan,
20 edotecarin, ELLENCE[®] or PHARMORUBICIN[®] (epirubicin), etoposide, exatecan, 10-hydroxycamptothecin, gimatecan, lurtotecan, mitoxantrone, orathecin, pirarubicin, pixantrone, rubitecan, sobuzoxane, SN-38, tafluposide, topotecan and the like.

- Antibodies include AVASTIN[®] (bevacizumab), CD40-specific antibodies, chTNT-1/B, denosumab, ERBITUX[®] (cetuximab), HUMAX-CD4[®] (zanolimumab),
25 IGF1R-specific antibodies, lintuzumab, PANOREX[®] (edrecolomab), RENCAREX[®] (WX G250), RITUXAN[®] (rituximab), ticilimumab, trastuzimab and the like.

Hormonal therapies include ARIMIDEX[®] (anastrozole), AROMASIN[®] (exemestane), arzoxifene, CASODEX[®] (bicalutamide), CETROTIDE[®] (cetorelix),

degarelix, deslorelin, DESOPAN[®] (trilostane), dexamethasone, DROGENIL[®],
 (flutamide), EVISTA[®] (raloxifene), AFEMA[™] (fadrozole), FARESTON[®] (toremifene),
 FASLODEX[®] (fulvestrant), FEMARA[®] (letrozole), formestane, glucocorticoids,
 HECTOROL[®] (doxercalciferol), RENAGEL[®] (sevelamer carbonate), lasofoxifene,
 5 leuprolide acetate, MEGACE[®] (megesterol), MIFEPREX[®] (mifepristone),
 NILANDRON[™] (nilutamide), NOLVADEX[®] (tamoxifen citrate), PLENAXIS[™]
 (abarelix), prednisone, PROPECIA[®] (finasteride), rilostane, SUPREFACT[®] (buserelin),
 TRELSTAR[®] (luteinizing hormone releasing hormone (LHRH)), VANTAS[®] (Histrelin
 implant), VETORYL[®] (trilostane or modrastane), ZOLADEX[®] (fosreltin, goserelin) and
 10 the like.

Deltoids and retinoids include seocalcitol (EB1089, CB1093), lexacalcitrol
 (KH1060), fenretinide, PANRETIN[®] (aliretinoin), ATRAGEN[®] (liposomal tretinoin),
 TARGRETIN[®] (bexarotene), LGD-1550 and the like.

PARP inhibitors include ABT-888, olaparib, KU-59436, AZD-2281, AG-014699,
 15 BSI-201, BGP-15, INO-1001, ONO-2231 and the like.

Plant alkaloids include, but are not limited to, vincristine, vinblastine, vindesine,
 vinorelbine and the like.

Proteasome inhibitors include VELCADE[®] (bortezomib), MG132, NPI-0052,
 PR-171 and the like.

20 Examples of immunologicals include interferons and other immune-enhancing
 agents. Interferons include interferon alpha, interferon alpha-2a, interferon alpha-2b,
 interferon beta, interferon gamma-1a, ACTIMMUNE[®] (interferon gamma-1b), or
 interferon gamma-n1, combinations thereof and the like. Other agents include
 ALFAFERONE[®], (IFN- α), BAM-002 (oxidized glutathione), BEROMUN[®]
 25 (tasonermin), BEXXAR[®] (tositumomab), CAMPATH[®] (alemtuzumab), CTLA4
 (cytotoxic lymphocyte antigen 4), decarbazine, denileukin, epratuzumab,
 GRANOCYTE[®] (lenograstim), lentinan, leukocyte alpha interferon, imiquimod,
 MDX-010 (anti-CTLA-4), melanoma vaccine, mitumomab, molgramostim,

MYLOTARG™ (gemtuzumab ozogamicin), NEUPOGEN® (filgrastim), OncoVAC-CL, OVAREX® (oregovomab), pentumomab (Y-muHMFG1), PROVENGE® (sipuleucel-T), sargaramostim, sizofilan, teceleukin, THERACYS® (Bacillus Calmette-Guerin), ubenimex, VIRULIZIN® (immunotherapeutic, Lorus Pharmaceuticals), Z-100 (Specific
5 Substance of Maruyama (SSM)), WF-10 (Tetrachlorodecaoxide (TCDO)), PROLEUKIN® (aldesleukin), ZADAXIN® (thymalfasin), ZENAPAX® (daclizumab), ZEVALIN® (90Y-Ibritumomab tiuxetan) and the like.

Biological response modifiers are agents that modify defense mechanisms of living organisms or biological responses, such as survival, growth, or differentiation of
10 tissue cells to direct them to have anti-tumor activity and include include krestin, lentinan, sizofiran, picibanil PF-3512676 (CpG-8954), ubenimex and the like.

Pyrimidine analogs include cytarabine (ara C or Arabinoside C), cytosine arabinoside, doxifluridine, FLUDARA® (fludarabine), 5-FU (5-fluorouracil), floxuridine, GEMZAR® (gemcitabine), TOMUDEX® (ratitrexed), TROXATYL™ (triacytyluridine
15 troxacinabine) and the like.

Purine analogs include LANVIS® (thioguanine) and PURI-NETHOL® (mercaptapurine).

Antimitotic agents include batabulin, epothilone D (KOS-862), N-(2-((4-hydroxyphenyl)amino)pyridin-3-yl)-4-methoxybenzenesulfonamide, ixabepilone (BMS
20 247550), paclitaxel, TAXOTERE® (docetaxel), PNU100940 (109881), patupilone, XRP-9881 (larotaxel), vinflunine, ZK-EPO (synthetic epothilone) and the like.

Compounds of this invention can also be used as radiosensitizers that enhance the efficacy of radiotherapy. Examples of radiotherapy include external beam radiotherapy, teletherapy, brachtherapy and sealed, unsealed source radiotherapy and the
25 like.

Additionally, compounds having Formula I may be combined with other chemotherapeutic agents such as ABRAXANE™ (ABI-007), ABT-100 (farnesyl transferase inhibitor), ADVEXIN® (Ad5CMV-p53 vaccine), ALTOCOR® or MEVACOR® (lovastatin), AMPLIGEN® (poly I:poly C12U, a synthetic RNA),

APTOSYN[®] (exisulind), AREDIA[®] (pamidronic acid), arglabin, L-asparaginase,
 atamestane (1-methyl-3,17-dione-androsta-1,4-diene), AVAGE[®] (tazarotene), AVE-8062
 (combreastatin derivative) BEC2 (mitumomab), cachectin or cachexin (tumor necrosis
 factor), canvaxin (vaccine), CEAVAC[®] (cancer vaccine), CELEUK[®] (celmoleukin),
 5 CEPLINE[®] (histamine dihydrochloride), CERVARIX[®] (human papillomavirus
 vaccine), CHOP[®] (C: CYTOXAN[®] (cyclophosphamide); H: ADRIAMYCIN[®]
 (hydroxydoxorubicin); O: Vincristine (ONCOVIN[®]); P: prednisone), CYPAT[™]
 (cyproterone acetate), combrestatin A4P, DAB(389)EGF (catalytic and translocation
 domains of diphtheria toxin fused via a His-Ala linker to human epidermal growth factor)
 10 or TransMID-107R[™] (diphtheria toxins), dacarbazine, dactinomycin, 5,6-
 dimethylxanthenone-4-acetic acid (DMXAA), eniluracil, EVIZON[™] (squalamine
 lactate), DIMERICINE[®] (T4N5 liposome lotion), discodermolide, DX-8951f (exatecan
 mesylate), enzastaurin, EPO906 (epithilone B), GARDASIL[®] (quadrivalent human
 papillomavirus (Types 6, 11, 16, 18) recombinant vaccine), GASTRIMMUNE[®],
 15 GENASENSE[®], GMK (ganglioside conjugate vaccine), GVAX[®] (prostate cancer
 vaccine), halofuginone, histerelin, hydroxycarbamide, ibandronic acid, IGN-101, IL-13-
 PE38, IL-13-PE38QQR (cintredekin besudotox), IL-13-pseudomonas exotoxin,
 interferon- α , interferon- γ , JUNOVAN[™] or MEPACT[™] (mifamurtide), lonafarnib, 5,10-
 methylenetetrahydrofolate, miltefosine (hexadecylphosphocholine), NEOVASTAT[®] (AE-
 20 941), NEUTREXIN[®] (trimetrexate glucuronate), NIPENT[®] (pentostatin), ONCONASE[®]
 (a ribonuclease enzyme), ONCOPHAGE[®] (melanoma vaccine treatment), ONCOVAX[®]
 (IL-2 Vaccine), ORATHECIN[™] (rubitecan), OSIDEM[®] (antibody-based cell drug),
 OVAREX[®] MAb (murine monoclonal antibody), paditaxel, PANDIMEX[™] (aglycone
 saponins from ginseng comprising 20(S)protopanaxadiol (aPPD) and
 25 20(S)protopanaxatriol (aPPT)), panitumumab, PANVAC[®]-VF (investigational cancer
 vaccine), pegaspargase, PEG Interferon A, phenoxodiol, procarbazine, rebimastat,
 REMOVAB[®] (catumaxomab), REVLIMID[®] (lenalidomide), RSR13 (efaproxiral),
 SOMATULINE[®] LA (lanreotide), SORIATANE[®] (acitretin), staurosporine

(*Streptomyces staurospores*), talabostat (PT100), TARGRETIN[®] (bexarotene), TAXOPREXIN[®] (DHA-paclitaxel), TELCYTA[®] (canfosfamide, TLK286), temilifene, TEMODAR[®] (temozolomide), tesmilifene, thalidomide, THERATOPE[®] (STn-KLH), thymitaq (2-amino-3,4-dihydro-6-methyl-4-oxo-5-(4-pyridylthio)quinazoline dihydrochloride), TNFERADE[™] (adenovector: DNA carrier containing the gene for tumor necrosis factor- α), TRACLEER[®] or ZAVESCA[®] (bosentan), tretinoin (Retin-A), tetrandrine, TRISENOX[®] (arsenic trioxide), VIRULIZIN[®], ukrain (derivative of alkaloids from the greater celandine plant), vitaxin (anti- α v β 3 antibody), XCYTRIN[®] (motexafin gadolinium), XINLAY[™] (atrasentan), XYOTAX[™] (paclitaxel poliglumex), YONDELIS[®] (trabectedin), ZD-6126, ZINECARD[®] (dexrazoxane), ZOMETA[®] (zoledronic acid), zorubicin and the like.

Cationic-Based Lipid Encapsulation Systems (CaBLES) and Lipid-Based Particles

CaBLES comprise one or more non-cationic lipids, one or more cationic lipids and one or more polyethylene glycol (PEG)-lipid conjugates having Formula I.

Lipid-Based Particles of the present invention are defined as CaBLES which further comprise one or more therapeutic agent(s). These particles have mean diameter sizes of 50-300 nm, of which 50-250 nm is preferred and 50-200 nm is most preferred. Functional CaBLES effectively encapsulate nucleic acids, (e.g., single stranded or double stranded DNA, single stranded or double stranded RNA, RNAi, siRNA, and the like). Suitable nucleic acids include, but are not limited to, plasmids, antisense oligonucleotides, ribozymes as well as other poly- and oligonucleotides. In preferred embodiments, the nucleic acid encodes a product, e.g., a therapeutic product, of interest. The CaBLES of the present invention can be used to deliver the nucleic acid to a cell (e.g., a cell in a mammal) for, e.g., expression of the nucleic acid or for silencing of a target sequence expressed by the cell.

In some embodiments, the nucleic acid is a siRNA molecule that silences the gene of interest, with efficiencies from about 50-100%, and more preferably between about 80-100%.

In other embodiments, the therapeutic agents that can be delivered with CaBLES include RNA, antisense oligonucleotide, a DNA, a plasmid, a ribosomal RNA (rRNA), a

micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA (snRNA), chimeric nucleic acids, an antigen, fragments thereof, a protein, a peptide, small-molecules, or mixtures thereof. This invention describes delivery of RNA's such as small inhibitory RNA or microRNA. The siRNA can have varying
5 lengths (10-200 bps) and structures (hairpins, single/double strands, bulges, nicks/gaps, mismatches) and processed in the cell to provide active gene silencing. In certain embodiments of this invention, a double-stranded siRNA (dsRNA) can have the same number of nucleotides on each strand (blunt ends) or asymmetric ends (overhangs). The overhang of 1-2 nucleotides can be present on the sense and/or the antisense strand, as
10 well as present on the 5'- and/ or the 3'-ends of a given strand.

Suitable siRNA sequences can be identified using means known in the art (e.g., methods described in Elbashir, et al., *Nature* 411:494-498 (2001) and Elbashir, et al., *EMBO J.* 20: 6877-6888 (2001) are combined with rational design rules set forth in Reynolds et al., *Nature Biotech.* 22(3):326-330 (2004)). Further enhancing, isolating,
15 synthesizing and generating of the siRNA can be done by various methods known in the art, (see, e.g., Elbashir, et al., *EMBO J.* 20: 6877-6888 (2001); Elbashir, et al., *Genes Dev.* 15:188 (2001); Nykanen, et al., *Cell* 107:309 (2001)) or may lack overhangs (i.e., to have blunt ends): and Gubler & Hoffman, *Gene* 25:263-269 (1983); Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); *Current Protocols in Molecular*
20 *Biology* (Ausubel et al., eds., 1994), as are PCR methods (see U.S. Pat. Nos. 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis et al., eds, 1990)).

Non-cationic lipids have a neutral charge or an anionic charge at physiological pH. A neutral lipid, also known as a "helper lipid," has no net charge at physiological pH.
25 These lipids can also be zwitterionic.

Polyethylene glycol (PEG)-lipid conjugates are used to minimize particle aggregation in solution, provide increased in vivo serum circulation, and enhance distribution of nanoparticles to organs, tissues, cell types, and tumors of interest. These shielding lipids consist of a lipid portion linked to a "PEG" portion via carbamate, ester,
30 amide, ether, amine, thioether, or dithiol linkages. "PEG" is a polyethylene glycol consisting of repeating C₂H₄O units with an average molecular weight between 500 to

10,000 daltons and may be substituted by alkoxy, acyl, alkyl, or aryl. Additionally, the PEG can be substituted at its terminus with one or more of the following functional groups: hydroxy, methoxy, primary, secondary, or tertiary amine, thiol, thioether, thiopyridyl, dithiol, maleimide, or ester. Particular polyethylene glycol (PEG)-lipid

5 conjugates of this invention are as described in Formula I and include 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl

3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-

hexatetracontaioxanonatriacontahect-1-ylcarbamate,

10 2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl

3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-

hexatetracontaioxanonatriacontahect-1-ylcarbamate,

2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl

15 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-

hexatetracontaioxanonatriacontahect-1-ylcarbamate,

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,

20 92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaioxaoctatriacontahectanamidopropane-1,3-diyl ditetradecanoate,

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,

92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

25 hexatetracontaioxaoctatriacontahectanamidopropane-1,3-diyl dipalmitate,

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,

92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaioxaoctatriacontahectanamidopropane-1,3-diyl distearate,

30 N-(2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl)-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,

- 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
 hexatetracontaoxanonatriacontahectan-139-amide
 N-(2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl)-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 5 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
 hexatetracontaoxanonatriacontahectan-139-amide, and
 N-(2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl)-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
 10 hexatetracontaoxanonatriacontahectan-139-amide. Polyethylene glycol (PEG)-lipid
 conjugates that are useful for the practice of this invention include, but are not limited to,
 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 15 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 20 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate,
 2-
 25 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
 hexatetracontaoxaoctatriacontahectanamidopropane-1,3-diyl ditetradecanoate,
 2-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 30 92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
 hexatetracontaoxaoctatriacontahectanamidopropane-1,3-diyl dipalmitate,

- 2-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
hexatetracontaaoctatriacontahectanamidopropane-1,3-diyl distearate,
- 5 N-(2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl)-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
hexatetracontaaoxanonatriacontahectan-139-amide
N-(2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl)-
- 10 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
hexatetracontaaoxanonatriacontahectan-139-amide, and
N-(2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl)-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
- 15 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
hexatetracontaaoxanonatriacontahectan-139-amide, 1,2-distearoyl-sn-glycerol-
methoxypolyethyleneglycol-750, 1,2-dimyristoyl-sn-glycerol-
methoxypolyethyleneglycol-750, 1,2-dipalmitoyl-sn-glycerol-
methoxypolyethyleneglycol-750, poly(oxy-1,2-ethanediyl)-2000- α -(3 β)-cholest-5-en-3-
20 yl-omega-hydroxy, 1,2-dipalmitoyl-sn-glycerol-methoxypolyethyleneglycol-5000,
poly(oxy-1,2-ethanediyl)-5000- α -(3 β)-cholest-5-en-3-yl-omega-hydroxy, (2S,3R,E)-3-
hydroxy-2-stearamidooctadec-4-enyl polyethyleneglycol-2000 methyl ether succinate,
(2S,3R,E)-3-hydroxy-2-icosanamidooctadec-4-enyl polyethyleneglycol-2000 methyl
ether succinate, N-(2,3-dimyristyloxypropyl)carbamate polyethyleneglycol-2000 methyl
25 ether, N-(carbonylmethoxypolyethyleneglycol-750)-1,2-dimyristoyl-sn-glycero-
phosphatidylethanolamine, N-(carbonyl-methoxypolyethyleneglycol-750)-1,2-distearoyl-
sn-glycero-3-phosphoethanolamine, N-(carbonyl-methoxypolyethyleneglycol-750)-1,2-
dipalmitoyl-sn-glycero-3-phosphoethanolamine, N-(carbonyl-
methoxypolyethyleneglycol-2000)-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine,
30 N-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-dipalmitoyl-sn-glycero-3-
phosphoethanolamine, N-(carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-

glycero-3-phosphoethanolamine, N-(carbonyl-methoxypolyethyleneglycol-2000)-dioleoyl-phosphatidylethanolamine, 1,2-distearoyl-sn-glycerol-methoxypolyethyleneglycol-2000, 1,2-dimyristoyl-sn-glycerol-methoxypolyethyleneglycol-2000, 1,2-dipalmitoyl-sn-glycerol-methoxypolyethyleneglycol-2000, mPEG-2000-cholesterol, octanoyl-mPEG-2000-ceramide, palmitoyl-mPEG-2000-ceramide, N-(carbonyl-methoxypolyethyleneglycol-5000)-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine, N-(carbonyl-methoxypolyethyleneglycol-5000)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, N-(carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-sn-glycerol-methoxypolyethyleneglycol-5000, 1,2-dipalmitoyl-sn-glycerol-methoxypolyethyleneglycol-5000, 1,2-distearoyl-sn-glycerol-methoxypolyethyleneglycol-5000, mPEG-5000-cholesterol, octanoyl-mPEG-5000-ceramide, palmitoyl-mPEG-5000-ceramide and mixtures thereof.

In some instances it may be desirable for the CaBLES and/or Lipid Based Particles to target using targeting moieties that are specific to a cell type or tissue. Targeting of liposomes using a variety of targeting moieties, such as ligands, cell surface receptors, glycoproteins, vitamins, (e.g., riboflavin) and monoclonal antibodies, has been previously described (see, e.g., U.S. Pat. Nos. 4,957,773 and 4,603,044). The targeting moieties can comprise the entire protein or fragments thereof. In one aspect, the targeting moiety is a small protein, or peptide. In another aspect, the targeting moiety is a small-molecule.

Cationic lipids are those having one or more moieties that are positively charged at a physiologically relevant pH, typically between 4-8. Examples of cationic lipids that are useful for the practice of this invention include, but are not limited to, N,N-dioleoyl-N,N-dimethylammonium chloride, DC-Chol; 1,3-dioleoyloxy-2-(6-carboxyspermyl)-propyl amide, dioctadecylamidoglycyl spermine, N,N-distearyl-N,N-dimethylammonium bromide, N-(2,3-dioleoyloxy)propyl)-N,N-dimethylammonium chloride, 1,2-dioleoyl-3-trimethylammonium-propane chloride, 1,2-dilinooyl-3-dimethylammonium-propane, N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride, 1,2-dioleoyl-3-dimethylammonium propane, 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane; didodecyltrimethylammonium bromide, dioleoyloxy-N-(2-sperminecarboxamido)ethyl)-

N,N-dimethyl-1-propanaminiumtrifluoroacetate, 1,2-dimyristyloxypropyl-3-dimethyl-
 hydroxyethyl ammonium bromide, 1,2-dioleoylcarbonyl-3-dimethylammoniumpropane,
 tetramethyltetrapalmitoyl spermine, tetramethyltetraoleyl spermine, tetramethyldioleyl
 spermine, tetramethyltetramyristyl spermine, tetramethyltetralauryl spermine, 1-(2-
 5 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)pyrrolidine; N,N-dimethyl-N-(2-((9Z,12Z)-octadeca-9,12-
 dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(3-(1H-
 imidazol-1-yl)propyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-
 9,12-dienyloxy)methyl)ethyl)amine; 1-methyl-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-
 10 1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)piperazine; 4-(2-((9Z,12Z)-octadeca-
 9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)morpholine; N-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N'-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 15 dienyloxy)methyl)ethyl)ethane-1,2-diamine; N-(2-(4-methylpiperazin-1-yl)ethyl)-N-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)amine; N-(2-(1H-imidazol-4-yl)ethyl)-N-(2-((9Z,12Z)-octadeca-
 9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N,N-
 dimethyl-N-(3-(4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 20 dienyloxy)methyl)ethyl)piperazin-1-yl)propyl)amine; 1,3-bis((9Z,12Z)-octadeca-9,12-
 dienyloxy)propan-2-amine; N-((1-methylpiperidin-4-yl)methyl)-N-(2-((9Z,12Z)-
 octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-
 (2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; N-methyl-N-(2-
 25 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N-(3-((4-methylpiperazin-1-
 yl)methyl)benzyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-
 9,12-dienyloxy)methyl)ethyl)amine; N-methyl-N-((1-methylpiperidin-4-yl)methyl)-N-(2-
 ((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 30 dienyloxy)methyl)ethyl)amine; N,N,N'-trimethyl-N'-(2-((9Z,12Z)-octadeca-9,12-
 dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)propane-1,3-diamine; N-

methyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; 1-(2-(1H-imidazol-
 1-yl)ethyl)-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-
 dienyloxy)methyl)ethyl)piperazine; N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-
 5 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-((2-pyrrolidin-1-ylpyridin-3-
 yl)methyl)amine; (9Z,9'Z,12Z,12'Z)-2-(4-methylpiperazin-1-yl)propane-1,3-diyl
 dioctadeca-9,12-dienoate; (9Z,9'Z,12Z,12'Z)-2-(3-(pyrrolidin-1-yl)propylamino)propane-
 1,3-diyl dioctadeca-9,12-dienoate; 1-methyl-4-(3-((9Z,12Z)-octadeca-9,12-dienyloxy)-2-
 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)piperazine; 1-(3-((9Z,12Z)-octadeca-
 10 9,12-dienyloxy)-2-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)pyrrolidine; N-(3-
 aminopropyl)-N'-{3-[(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-[(9Z,12Z)-octadeca-
 9,12-dienyloxy]methyl}ethyl)amino]propyl}butane-1,4-diamine; N-(3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl)-N-(3-
 pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N-(3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-
 15 {[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl)amine; 3-[(9Z,12Z)-octadeca-9,12-
 dienyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl 2-
 (diethylamino)ethylcarbamate; 3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]methyl}propyl 2-pyrrolidin-1-ylethylcarbamate; 3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl 2-
 20 (dimethylamino)ethylcarbamate; 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]methyl}ethyl)-4-(2-pyrrolidin-1-ylethyl)piperazine; N-(2-[(9Z)-
 octadec-9-enyloxy]-1-[(9Z)-octadec-9-enyloxy]methyl}ethyl)-N-(3-pyrrolidin-1-
 ylpropyl)amine, 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-[(9Z,12Z)-octadeca-9,12-
 dienyloxy]methyl}ethyl)azetidine, 2-methyl-1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-
 25 {[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}ethyl)aziridine, 1-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}piperidine, 4-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}morpholine, N,N-diethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, N,N-dimethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 30 phenylpiperazine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 methylpiperazine, N-(2-methoxyethyl)-N-methyl-3,4-bis[(9Z,12Z)-octadeca-9,12-

dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-methoxyphenyl)piperazine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N',N'-trimethylethane-1,2-diamine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-methyl-N-(2-pyridin-2-ylethyl)amine, N-benzyl-N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-methylamine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-(4-fluorobenzyl)-N-methylamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-fluorophenyl)piperazine, N-benzyl-N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-ethylamine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-ethyl-N',N'-dimethylethane-1,2-diamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpiperidin-4-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpyrrolidin-3-amine, N,N-bis(2-methoxyethyl)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-methoxypiperidine, 1-[(3R)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl]pyrrolidine, 1-[(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl]pyrrolidine, N-[(3R)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl]-N,N-diethylamine, N-[(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl]-N,N-diethylamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}pyrrolidine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)-N,N-diethylamine, 2-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)-1-methylpyrrolidine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)aziridine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)-4-methylpiperazine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)-N,N-dimethylamine, 4-(diethylamino)-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl (9Z,12Z)-octadeca-9,12-dienoate, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butoxy}ethyl)pyrrolidine, N,N-diethyl-N-(2-{2-[(8Z,11Z)-heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-yl}ethyl)amine, 1-[[[(9Z)-octadec-9-enoyloxy]methyl]-3-pyrrolidin-1-ylpropyl (9Z)-octadec-9-enoate, 1-{3,4-bis[(9Z)-octadec-9-enyloxy]butyl}pyrrolidine, 1-[[[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoyloxy]methyl]-3-pyrrolidin-1-ylpropyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate, (3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl 3-pyrrolidin-1-ylpropylcarbamate, 1-[3,4-

bis(octadecyloxy)butyl]pyrrolidine, 1-[3,4-bis(hexadecyloxy)butyl]pyrrolidine, 1-{3,4-bis[(9E)-hexadec-9-enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E)-octadec-9-enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E,12E)-octadeca-9,12-dienyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9Z,12Z,15Z)-octadeca-9,12,15-trienyloxy]butyl}pyrrolidine, N¹-{(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N³,N³-diethyl-beta-alaninamide, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-[3-(1H-imidazol-1-yl)propyl]amine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N',N'-trimethylpropane-1,3-diamine, 1-(1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}pyrrolidin-3-yl)-1H-imidazole, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-(3-pyrrolidin-1-ylpropyl)amine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N',N'-dimethylpropane-1,3-diamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}azetidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2-methylpyrrolidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2,5-dimethylpyrrolidine, are 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-1H-imidazole, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methyl-1,4-diazepane, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-phenylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-pyridin-2-ylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperidine, 4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)morpholine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-((2S)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-ethylpiperazine, N-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N-methyl-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine, N-(2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)ethyl)-N,N-dimethylamine, 1-((2S)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-(2-pyrrolidin-1-ylethyl)piperazine, 2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)pyrimidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N,N-diethylpyrrolidin-3-amine, 1-((9Z,12Z)-octadeca-9,12-

dienyloxy)-3-pyrrolidin-1-ylpropan-2-ol, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 1-({2-[(8Z,11Z)-heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-yl} methyl)pyrrolidine, 1-{2,3-bis[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]propyl}pyrrolidine, 1-{3-[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9E,12E)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2-[(9E,12E)-octadeca-9,12-dienyloxy]-3-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-[2,3-bis(tetradecyloxy)propyl]pyrrolidine, 1-[2,3-bis(octadecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-[2,3-bis(dodecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidin-3-ol, 1-{3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}-N,N-dimethylpyrrolidin-3-amine and 1-[3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-(tetradecyloxy)propyl]pyrrolidine, and mixtures thereof.

Lipid-Based Particles are a mixture of one or more PEG-lipid conjugates of Formula (I), one or more non-cationic lipids, one or more cationic lipids, and one or more therapeutic agents. Specific Lipid-Based Particles comprise the following lipid mixtures: cationic lipid(s) (about 2-60% by weight), non-cationic lipid(s) (about 5-90% by weight), and PEG-lipid conjugate(s) (about 0.1-20%).

Data

Tables 1 and 2
Representative Formulation of Lipid-Based Particles

25

Table 1

Therapeutic Agent	Mass (mg)	Vol (mL in water 10 mg/mL)
TetR-siRNA	3.0	0.3
	Mass (mg)	Vol (μL in ethanol 10 mg/mL)
Total Lipids	75	7.5

Table 2

	Wt%	Mass (mg)	Vol (μ L in ethanol, 10 mg/mL)
PEG-lipid conjugate	9	6.75	675
DSPC	14	10.5	1050
Cholesterol	33	24.75	2475
Cationic lipid	44	33	3300

Preparation of Lipid Mixture Solution

The lipid solution was prepared (10 mg/ml) by dissolving the lipid in 200 proof ethanol. The lipid mixture solution is prepared according to the above composition in Table 2.

Preparation of siRNA Solution

An siRNA (TetR_ODC_12, G.G.G.G.A.A.A.G.C.U.G.G.C.A.A.G.A.U.U.U.U SEQ ID NO. 1) (ThermoFisher) solution is prepared in a concentration of 10 mg/ml by dissolving 10 mg siRNA in 1 ml of DNase/RNase-free distilled water.

Preparation of Lipid-Based Particles

A round bottom flask was submerged into a 65°C water bath. Citrate buffer (37.5 ml) of pH 4.0 was pipetted into the flask. The solution was stirred by a magnetic stirring bar at a speed of 900 rpm. Both the pH 4.0 citrate buffer and the lipid solution were pre-warmed in the 65°C water bath for about 3 minutes. A siRNA solution (0.5 ml) was pipetted into the pH 4.0 citrate buffer. The 12.5 ml lipid mixture solution was injected through a 27 gauge needle into the citrate buffer in about 30 seconds. The needle tip was inserted into the solution during the injection. The resulting solution was stirred for 5 minutes at a speed of 900 rpm. The flask was pulled up from the water bath and a 50 ml pH 7.4 PBS buffer was added into the flask. The final solution was further mixed at a speed of 900 rpm for 5 minutes. For the diafiltration process, a dialysis filter (Millipore, 100K, Cat# PXB100C50) was used to remove ethanol in the above solution. When the volume was reduced to 20 ml during the initial diafiltration, 20 ml of pH 7.4 PBS was added to the sample solution. The diafiltration was continued until the volume was reduced to 20 ml. The diafiltration process was repeated 4 times. The volume of the sample solution was reduced to about 12 ml and pH 7.4 PBS was added to make the final

volume of 15 ml. The 15 ml solution was filtered sequentially through the 0.45 and 0.22 μm sterile PVDF membrane filters (Millipore) and immediately transferred into a sterile vial.

Analysis of Lipid-Based Particles

5 The siRNA concentrations were measured using Quanti-iT RiboGreen RNA reagent (Molecular Probes, (R11490)). Vesicle sizes were characterized by dynamic light scattering with a DynaPro™ Plate Reader (Wyatt Technology) in 96-well half-area UV plate (Corning) after diluting the formulation sample (20 μL) in phosphate buffered saline (80 μL) at a pH of about 7-8. A 1% agarose gel-based assay was used for analyzing
10 nuclease degradation and protection. Encapsulation efficiency (EE) was calculated using data obtained from a RiboGreen assay.

Ribogreen Assay for Measuring SiRNA Concentration and Encapsulation Efficiency of Lipid-Based Particles

RNA concentration and encapsulation efficiency were determined using a Quanti-
15 iT® Ribogreen RNA reagent and kit available from Invitrogen. The siRNA was released from the Lipid-Based Particle using one of the following reagents: ethanol, Triton X-100, or phenol/chloroform. The siRNA concentration is quantified using fluorescent reading at 480 nm/520 nm.

20

Particle Sizing Assay

Particle sizes and size distributions (PDI) were characterized by using dynamic light scattering (DLS). A DLS plate reader (Dynapro™, Wyatt Technology) was used for the DLS measurement. This DLS plate reader uses an 830 nm laser and the scattering
25 angle is 158°. It also can control temperature from 4°C to 70°C. A 96-well format was employed for the samples.

Samples for DLS analysis were prepared by mixing 20 μL of each sample stock solution with 80 μL PBS directly in the 96-well plate (#3697, Corning). Sample mixing was accomplished using a microplate shaker (Orbis, Mikura Ltd.). Plates were read at
30 20°C with an acquisition time of 50 seconds for each sample, and data was analyzed with Wyatt Technology's Dynamics V6 software. To rule out potential multiple scattering

artifacts, a second plate at 4-fold reduced sample concentrations was independently prepared by mixing 5 μ L stock solutions with 95 μ L PBS. Under our experimental conditions the results at the two concentrations were very similar, and the final reported result for each sample represents the average of values obtained from the two plates.

5

Table 3

Data Table Of Particle Size And Encapsulation Efficiency

Lipid-Based Particle No.	PEG-lipid Conjugate	Cationic Lipid	Particle Size (d/nm)	Encapsulation Efficiency (%)
1	Example 1	Example 10	129	96
2	Example 3	Example 10	164	97

In vivo Procedure to Determine Efficacy of CaBLES

The in vivo knockdown activities of formulations were tested using Abbott's positive readout system (MDA-TetR-Luc cells). Liver tumors were established by direct inoculation of tumor cells into the liver of SCID female mice (Charles River). 14 to 20 days later, the background bioluminescence of tumors were measured by IVIS Imaging System (Caliper Life Science) and the mice were signal-matched.

Formulated siRNAs were delivered through tail vein at 0.2mL per mouse, equivalent to 2.5mg/kg of siRNA. As a positive and negative control, TetR and non-target-composition (NTC) siRNAs were formulated in a benchmark formulations and included in the studies.

Mice were dosed at day 1 and 2, the bioluminescence were recorded on day 1 (before dosing) and day 4. The ratio of bioluminescence of day 4 vs day 1 was calculated for each animal and an increase indicates target knockdown.

Synthesis

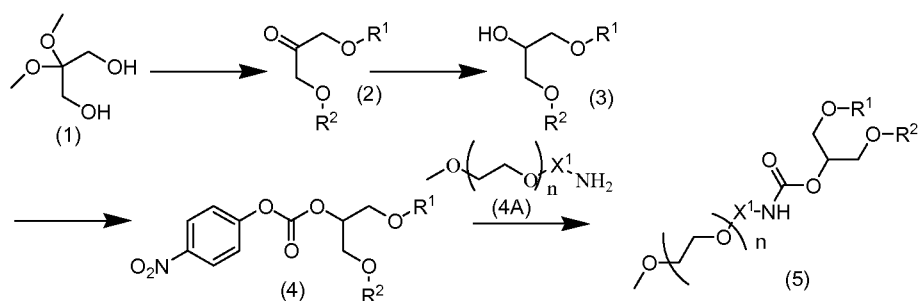
The following abbreviations have the meanings indicated: ADDP means 1,1'-(azodicarbonyl)dipiperidine; AD-mix- β means a mixture of (DHQD)₂PHAL, K₃Fe(CN)₆, K₂CO₃ and K₂SO₄; AIBN means 2,2'-azobis(2-methylpropionitrile); 9-BBN means 9-borabicyclo(3.3.1)nonane; Cp means cyclopentadiene; (DHQD)₂PHAL means hydroquinidine 1,4-phthalazinediyl diethyl ether; DBU means 1,8-diazabicyclo(5.4.0)undec-7-ene; DCC means dicyclohexylcarbodiimide; DIBAL means

diisobutylaluminum hydride; DIEA means diisopropylethylamine; DMAP means N,N-dimethylaminopyridine; DME means 1,2-dimethoxyethane; DMF means N,N-dimethylformamide; dmpe means 1,2-bis(dimethylphosphino)ethane; DMSO means dimethylsulfoxide; dppa means diphenylphosphoryl azide; dppb means 1,4-bis(diphenylphosphino)butane; dppe means 1,2-bis(diphenylphosphino)ethane; dppf means 1,1'-bis(diphenylphosphino)ferrocene; dppm means 1,1-bis(diphenylphosphino)methane; EDAC means 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; Fmoc means fluorenylmethoxycarbonyl; HATU means O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; HMPA means hexamethylphosphoramide; IPA means isopropyl alcohol; LDA means lithium diisopropylamide; LHMDs means lithium bis(hexamethyldisilylamide); MP-BH₃ means macroporus triethylammonium methylpolystyrene cyanoborohydride; LAH means lithium aluminum hydride; NCS means N-chlorosuccinimide; PyBOP means benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; TDA-1 means tris(2-(2-methoxyethoxy)ethyl)amine; TEA means triethylamine; TFA means trifluoroacetic acid; THF means tetrahydrofuran; NCS means N-chlorosuccinimide; NMM means N-methylmorpholine; NMP means N-methylpyrrolidine; PPh₃ means triphenylphosphine.

The following schemes are presented to provide what is believed to be the most useful and readily understood description of procedures and conceptual aspects of this invention. Compounds of this invention may be made by synthetic chemical processes, examples of which are shown herein. It is meant to be understood that the order of the steps in the processes may be varied, that reagents, solvents and reaction conditions may be substituted for those specifically mentioned, and that vulnerable moieties may be protected and deprotected, as necessary.

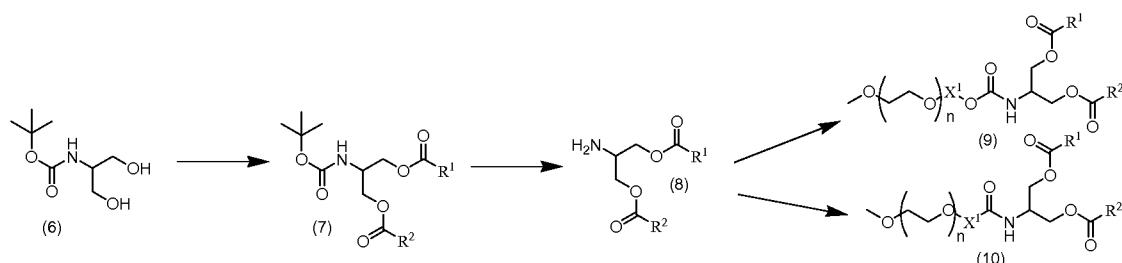
Schemes

Scheme 1



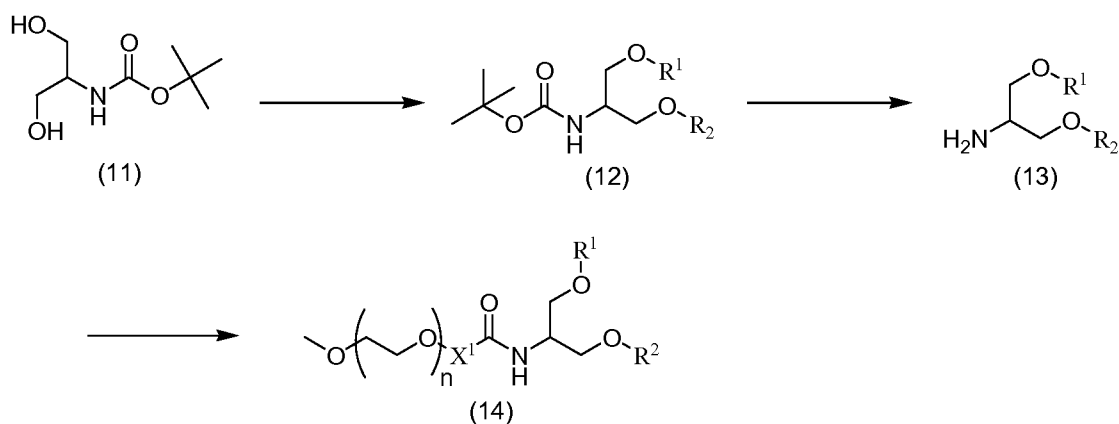
- 5 As shown in Scheme 1, 2,2-dimethoxypropane-1,3-diol (1) can be treated with sodium hydride, followed by R^1Br , to provide compounds of Formula (2). The reaction is typically performed in a solvent such as but not limited to toluene at low temperature before warming to room temperature. Compounds of Formula (3) can be prepared from compounds of Formula (2) by reacting the latter with a reducing agent such as but not
- 10 limited to sodium borohydride. The reaction is typically performed in a solvent such as but not limited to tetrahydrofuran, water, or mixtures thereof at ambient temperature. Compounds of Formula (4) can be prepared by reacting compounds of Formula (3) with 4-nitrophenyl carbonochloridate, in the presence of a base such as but not limited to triethylamine. The reaction is typically performed in a solvent such as but not limited to
- 15 dichloromethane at low temperature before warming to room temperature. Compounds of Formula (5), which are representative of compounds of Formula (I) wherein $R^1 = R^2$ and L^1 is drawn from left to right and is $NHC(O)O$, can be prepared by reacting compounds of Formula (4) with an appropriate PEGylation reagent of Formula (4A) in the presence of a base such as but not limited to triethylamine, or Hunig's base. The
- 20 reaction is typically conducted at ambient temperature in a solvent such as but not limited to dichloromethane.

Scheme 2



- As shown in Scheme 2, compounds of Formula (7), wherein $R^1 = R^2$, can be prepared by reacting compounds of Formula (6) with a compound of Formula $R^3\text{COOH}$, wherein $R^1 = R^2$ and $R^2 = R^3$. The reaction can be performed using coupling conditions known by those skilled in the art and readily available in the literature. Compounds of Formula (7) can be reacted with an acid such as trifluoroacetic acid to provide compounds of Formula (8). The reaction is typically performed at ambient temperature in a solvent such as but not limited to dichloromethane. Compounds of Formula (9) and (10), which are representative of compounds of Formula (I) wherein $R^1 = R^2$, can be prepared by reacting compounds of Formula (8) with an appropriate PEGylation reagent in the presence of a base such as but not limited to triethylamine, or Hunig's base. The reaction is typically conducted at ambient temperature in a solvent such as but not limited to dichloromethane.

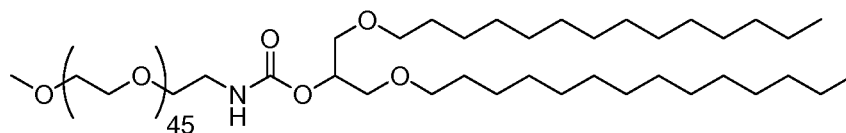
Scheme 3



As shown in Scheme 3, compounds of Formula (11) can be reacted with R^1Br , in the presence of a strong base such as sodium hydride, to provide a compound of Formula (12), wherein $R^1=R^2$. The reaction is typically conducted in a solvent such as but not limited to N,N-dimethylformamide at low temperature before warming to an elevated temperature. Compounds of Formula (12) can be reacted with an acid such as trifluoroacetic acid to provide compounds of Formula (13). The reaction is typically performed at ambient temperature in a solvent such as but not limited to dichloromethane. Compounds of Formula (14), which are representative of compounds of Formula (I) wherein $R^1=R^2$, can be prepared by reacting compounds of Formula (13) with an appropriate PEGylation reagent in the presence of a base such as but not limited to triethylamine, or Hunig's base. The reaction is typically conducted at ambient temperature in a solvent such as but not limited to dichloromethane.

The following examples are presented to provide what is believed to be the most useful and readily understood description of procedures and conceptual aspects of this invention. The exemplified compounds were named using ACD/ChemSketch Version 5.06 (05 June 2001, Advanced Chemistry Development Inc., Toronto, Ontario), or ChemDraw® Ver. 9.0.5 (CambridgeSoft, Cambridge, MA). Intermediates were named using ChemDraw® Ver. 9.0.5 (CambridgeSoft, Cambridge, MA).

Examples

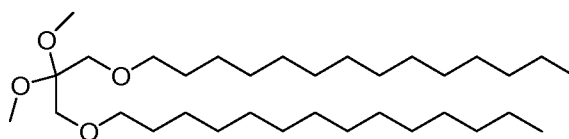


EXAMPLE 1

2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl

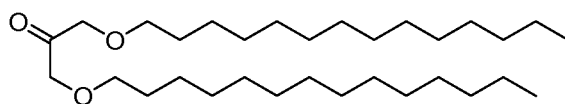
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hex

atetracontaoxanonatriacontahect-1-ylcarbamate



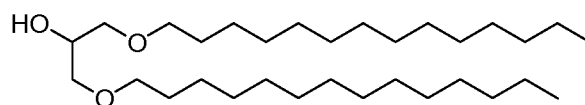
EXAMPLE 1A

To a solution of 2,2-dimethoxypropane-1,3-diol (1 g) in toluene (30 mL) at 0°C was added NaH (1.484 g). The mixture was stirred at room temperature for 1 hour. The mixture was cooled to 0°C, and 1-bromotetradecane (4.99 mL) was added. The mixture was heated at reflux for 2 hours. The mixture was cooled to 0°C, and ethanol was added until it became clear. The mixture was concentrated. The concentrate was taken up in dichloromethane and dried onto silica gel. The silica was loaded into an Analogix DASI module, and the product was isolated by flash chromatography (Analogix, SF65×200g, 2% ethyl acetate/hexanes for six column volumes, then 4% ethyl acetate/hexanes until major product eluted). MS (ESI) m/z 512 (M-CH₃+1).



EXAMPLE 1B

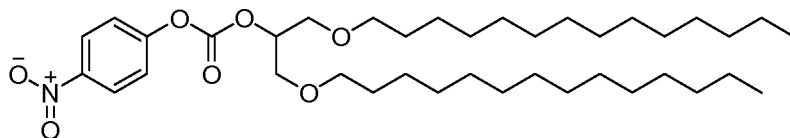
To a solution of 1-(2,2-dimethoxy-3-(tetradecyloxy)propoxy)tetradecane (2.2 g) in tetrahydrofuran (60 mL) was added 6N hydrogen chloride (5.55 mL). The mixture was stirred at room temperature overnight then concentrated. The concentrate was taken up in ethyl acetate, washed with saturated NaHCO₃, dried over Na₂SO₄, filtered, and concentrated. The concentrate was dissolved in dichloromethane and concentrated onto silica gel. The silica gel was loaded into an Analogix DASI module, and the product was isolated by flash chromatography (Analogix, SF65×200g, 2% ethyl acetate/hexanes for six column volumes, then 4% ethyl acetate/hexanes until the product eluted). MS (ESI) m/z 500.4 (M+18)⁺.



EXAMPLE 1C

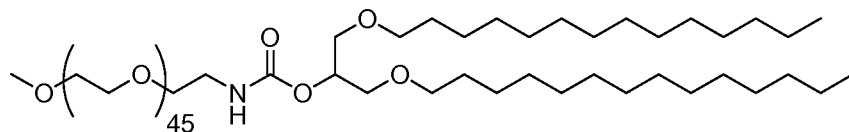
To a solution of 1,3-bis(tetradecyloxy)propan-2-one (0.68 g) in tetrahydrofuran (13 mL) at 0°C was added sodium borohydride (0.085 g) and water (0.867 mL). The mixture was stirred at room temperature for 1 hour, cooled to 0°C, and quenched with 1N HCl. The mixture was extracted with ethyl acetate. The extract was dried over Na₂SO₄,

filtered and concentrated. The concentrate was purified by flash chromatography (1:5 ethyl acetate/hexanes). MS (ESI) m/z 484 ($M+1$)⁺, 502 ($M+18$)⁺.



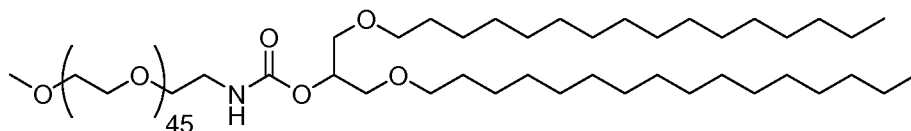
EXAMPLE 1D

5 To a solution of 1,3-bis(tetradecyloxy)propan-2-ol (0.3 g) in dichloromethane (3 mL) at 0°C were added triethylamine (0.129 mL) and 4-nitrophenyl carbonochloridate (0.137 g). The mixture was stirred at room temperature overnight and concentrated. The concentrate was purified by flash chromatography (1:10 ethyl acetate/hexanes). ¹H NMR (300 MHz, CDCl₃) δ 8.24-8.30 (m, 2H), 7.37-7.42 (m, 2H), 5.06-5.13 (m, 1H), 3.67 (d, 10 J=5.16 Hz, 4H), 3.41-3.55 (m, 4H), 1.55-1.60 (m, 4H), 1.19-1.38 (m, 44H), 0.85-0.90 (m, 6H).



EXAMPLE 1E

To a solution of CH₃O-PEG2000-NH₂ (12 2000-2 Rapp Polymere, 0.2 g) in 15 dichloromethane (1 mL) were added 1,3-bis(tetradecyloxy)propan-2-yl 4-nitrophenyl carbonate (0.195 g) and triethylamine (0.015 g). The mixture was stirred at room temperature overnight. The mixture was directly purified by flash chromatography (5-20% methanol/dichloromethane). ¹H NMR (300 MHz, CDCl₃) δ 3.53-3.66 (m, 180H), 3.32-3.49 (m, 9H), 3.38 (s, 3H), 1.51-1.59 (m, 4H), 1.21-1.35 (m, 44H), 0.86-0.90 (m, 20 6H); MS (MALDI) m/z 2549.



EXAMPLE 2

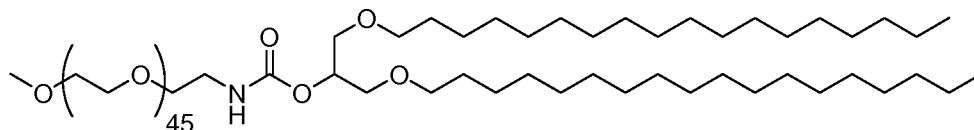
2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl

3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexat

5

etracontaoxanonatriacontahect-1-ylcarbamate

This EXAMPLE was prepared as described in EXAMPLE 1, substituting hexadecyl methanesulfonate for 1-bromotetradecane in EXAMPLE 1A. ^1H NMR (300 MHz, CDCl_3) δ 3.54-3.66 (m, 180H), 3.32-3.49 (m, 9H), 3.38 (s, 3H), 1.51-1.59 (m, 4H), 1.21-1.36 (m, 48H), 0.86-0.90 (m, 6H); MS (MALDI) m/z 2614.



10

EXAMPLE 3

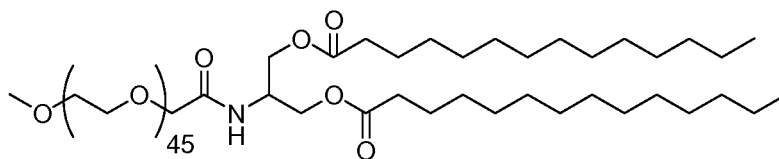
2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl

3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexat

15

etracontaoxanonatriacontahect-1-ylcarbamate

This EXAMPLE was prepared as described in EXAMPLE 1 substituting octadecyl methanesulfonate for 1-bromotetradecane in EXAMPLE 1A. ^1H NMR (300 MHz, CDCl_3) δ 3.52-3.66 (m, 180H), 3.32-3.49 (m, 9H), 3.38 (s, 3H), 1.51-1.59 (m, 4H), 1.21-1.36 (m, 52H), 0.86-0.90 (m, 6H); MS (MALDI) m/z 2557.



20

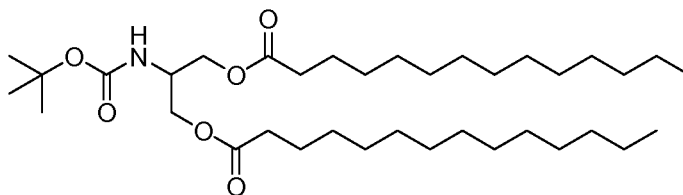
EXAMPLE 4

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

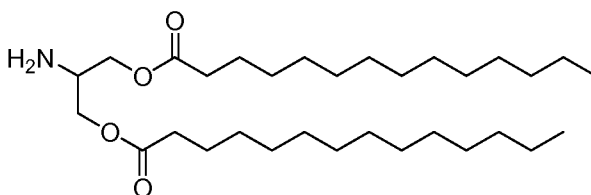
25

hexatetracontaoxaoctatriacontahectanamidopropane-1,3-diyl ditetradecanoate



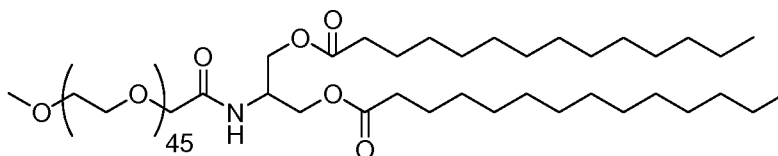
EXAMPLE 4A

To a solution of tetradecanoic acid (1.051 g) in dichloromethane (10 mL) at 0°C were added tert-butyl 1,3-dihydroxypropan-2-ylcarbamate (0.40 g), 4-(dimethylamino)pyridine (0.562 g), N-methylmorpholine (1.150 mL), and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (0.882 g). The mixture was stirred at room temperature overnight. The mixture was partitioned between water and dichloromethane. The aqueous layer was extracted with dichloromethane. The extract were dried over Na₂SO₄, filtered, and concentrated. The concentrate was purified by flash chromatography (1:10 ethyl acetate/hexanes). MS (ESI) m/z 512.4 (M-CO₂-tert-butyl+1)⁺.



EXAMPLE 4B

To a solution of 2-(tert-butoxycarbonylamino)propane-1,3-diyl ditetradecanoate in dichloromethane (10 mL) was added trifluoroacetic acid. The mixture was stirred at room temperature for 2 hours then concentrated. The concentrate was purified by flash chromatography. MS (ESI) m/z 512.4 (M+1)⁺.

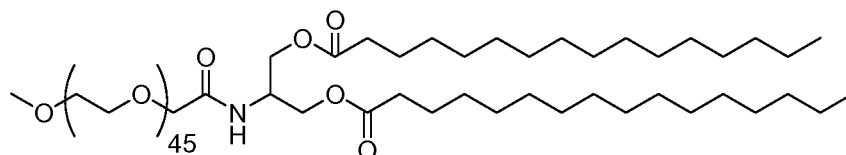


EXAMPLE 4C

To a flask was charged with mPEG2000-SCM (Laysan, 0.2 g) and 2-aminopropane-1,3-diyl ditetradecanoate (0.077 g) was added dichloromethane (2 mL).

The mixture was stirred at room temperature overnight and concentrated. The concentrate was purified by flash chromatography (5-20% methanol/dichloromethane).

^1H NMR (300 MHz, CDCl_3) δ 4.11-4.21 (m, 4H), 4.01 (s, 2H), 3.53-3.68 (m, 180H), 3.39-3.42 (m, 1H), 3.38 (s, 3H), 2.31 (t, $J=7.46$ Hz, 4H), 1.57-1.64 (m, 4H), 1.20-1.37 (m, 40H), 0.85-0.90 (m, 6H); MS (MALDI) m/z 2632.



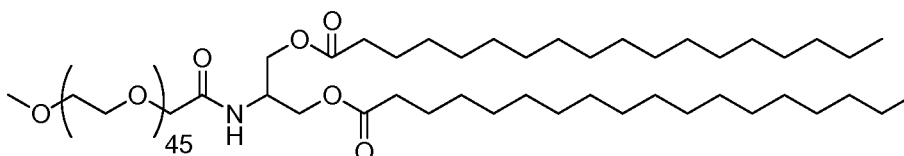
EXAMPLE 5

2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaoxaoctatriacontahectanamidopropane-1,3-diyl dipalmitate

This EXAMPLE was prepared as described in EXAMPLE 4, substituting hexadecanoic acid for tetradecanoic acid in EXAMPLE 4A. ^1H NMR (300 MHz, CDCl_3) δ 4.10-4.21 (m, 4H), 4.01 (s, 2H), 3.53-3.69 (m, 180H), 3.39-3.42 (m, 1H), 3.38 (s, 3H), 2.31 (t, $J=7.63$ Hz, 4H), 1.56-1.63 (m, 4H), 1.20-1.33 (m, 44H), 0.85-0.91 (m, 6H); MS (MALDI) m/z 2732.



EXAMPLE 6

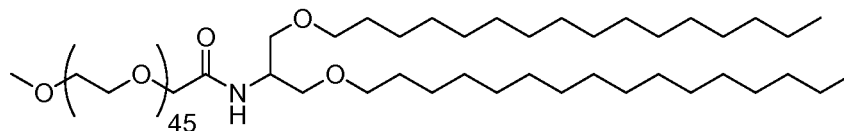
2-

2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-

hexatetracontaoxaoctatriacontahectanamidopropane-1,3-diyl distearate

This EXAMPLE was prepared as in EXAMPLE 4, substituting octadecanoic acid for tetradecanoic acid in EXAMPLE 4A. ^1H NMR (300 MHz, CDCl_3) δ 4.10-4.21 (m, 4H), 4.01 (s, 2H), 3.53-3.69 (m, 180H), 3.39-3.42 (m, 1H), 3.38 (s, 3H), 2.31 (t, $J=7.63$

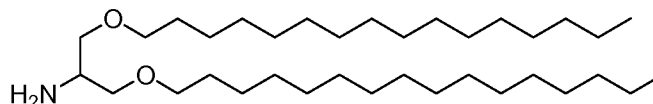
Hz, 4H), 1.57-1.63 (m, 4H), 1.21-1.33 (m, 48H), 0.85-0.90 (m, 6H); MS (MALDI) m/z 2832.



5

EXAMPLE 7

N-(2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl)-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-h
exatetracontaoxanonatriacontahectan-139-amide



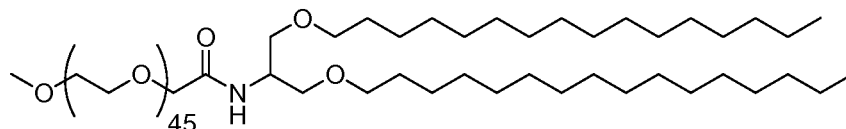
10

EXAMPLE 7A

In a 100 mL round-bottomed flask was added N-Boc-serinol (1,1-dimethylethyl (2-hydroxy-1-(hydroxymethyl)ethyl)carbamate) (2.0 g) and sodium hydride (1.255 g) in N,N-dimethylformamide (50 mL). The mixture was cooled using an ice/water bath, and
15 1-bromohexadecane (7.98 g) was added to it. The mixture was heated at 70°C overnight, then cooled to room temperature. The mixture was cooled to 0°C and quenched with a few drops of cold water. The mixture was diluted with saturated ammonium chloride (50 mL). The aqueous layer was extracted with ethyl acetate, and the extract was washed with brine, dried over Na₂SO₄, and concentrated. The concentrate was added to a silica
20 gel column and was eluted with ethyl acetate/hexane (1:9). The product, tert-butyl 1,3-bis(hexadecyloxy)propan-2-ylcarbamate, was directly used for the next step.

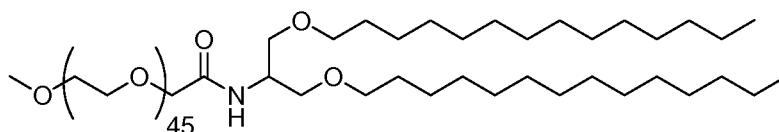
In a 100 mL round-bottomed flask was added tert-butyl 1,3-bis(hexadecyloxy)propan-2-ylcarbamate (5.0 g) and CH₂Cl₂ (40 mL). Trifluoroacetic acid (20 mL) was then added dropwise. The mixture was stirred under nitrogen for 3
25 hours and concentrated. The concentrate was added to a silica gel column and eluted with CH₂Cl₂/methanol (9:1). The product was dried under vacuum. ¹H NMR (300 MHz,

CDCl₃) δ 3.53-3.63 (m, 4H), 3.42-3.46 (t, 4H), 3.23 (m, 1H), 2.92-2.97 (m, 2H), 1.53-1.64 (m, 4H), 1.18-1.40 (m, 52H), 0.86-0.90 (t, 6H). MS (ESI) m/z 540.6 (M+1)⁺.



EXAMPLE 7B

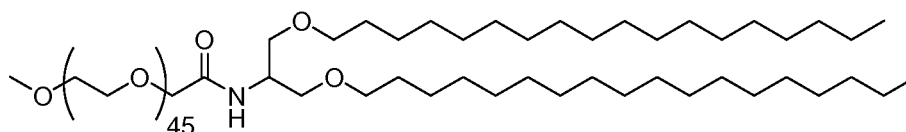
5 Into a 40 mL glass vial was added 1,3-bis(hexadecyloxy)propan-2-amine (1.75 g) and mPEG2000-SCM (Laysan, 0.25 g, 1.081 mmol) in CH₂Cl₂ (10 mL). Triethylamine (0.50 mL) was added dropwise. The reaction solution was stirred under nitrogen for one day. The crude product was added to a silica gel column and was eluted with CH₂Cl₂ / methanol (9:1). The product was dried under vacuum. ¹H NMR (300 MHz, CDCl₃) δ
 10 4.17- 4.18 (m, 1H), 4.14 (s, 2H), 3.86 -3.88 (m, 4H), 3.74-3.76 (t, 4H), 3.61-3.71 (m, 180H), 3.38 (s, 3H), 1.51-1.59 (m, 4H), 1.23-1.32 (m, 56H), 0.86-0.90 (m, 6H); MS (MALDI) m/z 2700.



EXAMPLE 8

15 N-(2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl)-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137
 -hexatetracontaoxanonatriacontahectan-139-amide

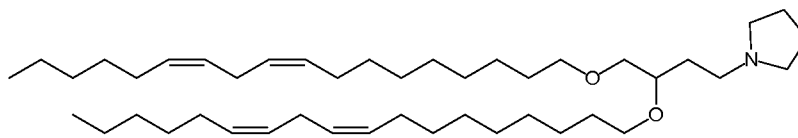
This EXAMPLE was prepared as described in EXAMPLE 7, substituting 1-
 20 bromotetradecane for 1-bromohexadecane in EXAMPLE 7A. ¹H NMR (300 MHz, CDCl₃) δ 4.18 (m, 1H), 4.10 (s, 2H), 3.86-3.89 (m, 4H), 3.72-3.75 (t, 4H), 3.61-3.71 (m, 180H), 3.38 (s, 3H), 1.50-1.60 (m, 4H), 1.24-1.30 (m, 48H), 0.86-0.90 (m, 6H); MS (MALDI) m/z 2400.



EXAMPLE 9

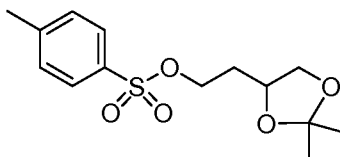
N-(2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl)-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-h
 5 exatetracontaoxanonatriacontahectan-139-amide

This EXAMPLE was prepared as described in EXAMPLE 7, substituting 1-
 bromooctadecane hexadecane for 1-bromotetradecane in EXAMPLE 7A. ¹H NMR (300
 MHz CDCl₃) δ 4.14- 4.20 (m, 1H), 4.08 (s, 2H), 3.86 -3.89 (t, 4H), 3.71-3.75 (m, 4H),
 3.61-3.70 (m, 180H), 3.38 (s, 3H), 1.50-1.56 (m, 4H), 1.20-1.30 (m, 64H), 0.86-0.90 (m,
 10 6H); MS (MALDI) m/z 2900.



EXAMPLE 10

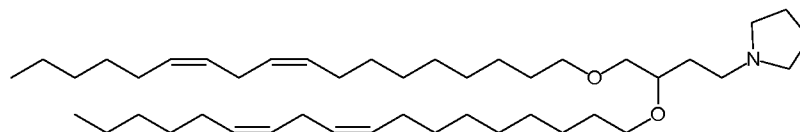
1-(3,4-bis((9Z,12Z)-octadeca-9,12-dienyloxy)butyl)piperidine



EXAMPLE 10A

2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl 4-methylbenzenesulfonate

2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanol (5 g) in dichloromethane (86 mL) at
 0°C was treated with TEA (6.9 g), para-toluenesulfonyl chloride (6.5 g) and
 4-DMAP (0.42 g), stirred overnight, quenched with saturated NH₄Cl and diluted with
 20 ethyl acetate. The extract was dried (Na₂SO₄), filtered, and concentrated. The
 concentrate was purified by flash column chromatography (0-100% ethyl
 acetate/hexanes, Analogix). ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, J=8.29Hz, 2H) 7.35
 (d, J=7.98Hz, 2H) 4.06-4.23 (m, 3H) 4.01 (dd, J=7.98, 6.14Hz, 1H) 3.51 (dd, J=8.13,
 6.90Hz, 1H) 2.45 (s, 3H) 1.82-1.98 (m, 2H) 1.31 (d, J=18.72Hz, 6H).



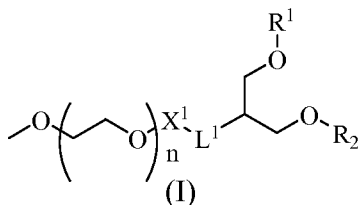
EXAMPLE 10B

1-(3,4-bis((9Z,12Z)-octadeca-9,12-dienyloxy)butyl)piperidine

- 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl 4-methylbenzenesulfonate (500 mg),
- 5 pyrrolidine (1-2 eq) and Hunig's base (2 eq) in dioxane (2.2 mL) was microwaved (Biotage Initiator) for 15 minutes at 140°C, treated with 4NHCl (4 mL) until acidic, stirred overnight at room temperature, treated with 6N NaOH until basic, diluted with water and extracted with chloroform. The extract was dried (Na₂SO₄), filtered and concentrated. The concentrate in toluene (0.3 M) was treated with NaH (5-10 eq), stirred
- 10 for 45 minutes, treated with (9Z,12Z)-octadeca-9,12-dienyl methanesulfonate (Nu-Check Prep, 2.5 eq), stirred at 80-90°C for 4 hours, treated with ethanol then ethyl acetate and water. The water was extracted with ethyl acetate, and the extract was dried (Na₂SO₄), filtered and concentrated. The concentrate was purified by flash column chromatography (0-100%ethyl acetate /hexanes, Analogix) to afford the title compound. ¹H NMR (300
- 15 MHz, CDCl₃) δ 5.25-5.45 (m, 8H) 3.51-3.63 (m, 1H) 3.33-3.51 (m, 6H) 2.77 (t, J=6.10Hz, 4H) 2.44-2.58 (m, 6H) 2.05 (q, J=6.55Hz, 8H) 1.48-1.82 (m, 10H) 1.20-1.44 (m, 34H) 0.84-0.96 (m, 6H).

WE CLAIM:

1. A polyethylene glycol (PEG)-lipid conjugate having Formula I



wherein

R^1 and R^2 are independently R^3 or $C(O)R^3$; or

R^1 and R^2 together are $C(R^3)_2$;

R^3 is C_8 - C_{24} -alkyl;

X^1 is C_1 - C_6 -alkyl;

L^1 is drawn from left to right and is $C(OCH_3)_2$, $NHC(O)$, $C(O)NH$, $OC(O)NH$, $NHC(O)O$, $NHC(O)NH$, $N(N)C(O)$, $C(O)N(N)$, SS , $NHC(O)L^2C(O)O$, $NHC(O)L^2C(O)NH$, $OC(O)L^2C(O)O$, $OC(O)L^2C(O)NH$, $C(O)O$, $OC(O)$, S , O , NH , $CH_2CH(=N)NHR^4C(O)$, $C(=NNHCH_3)R^4$, $C(OCH_3)_2CH_2$, $NHC(O)CH_2$, $C(O)NHCH_2$, $OC(O)NHCH_2$, $NHC(O)OCH_2$, $NHC(O)NHCH_2$, $N(N)C(O)CH_2$, $C(O)N(N)CH_2$, $SSCH_2$, $NHC(O)L^2C(O)OCH_2$, $NHC(O)L^2C(O)NHCH_2$, $OC(O)L^2C(O)OCH_2$, $OC(O)L^2C(O)NHCH_2$, $C(O)OCH_2$, $OC(O)CH_2$, SCH_2 , OCH_2 , $NHCH_2$, $CH_2CH(=N)NHR^4C(O)CH_2$ or $C(=NNHCH_3)R^4CH_2$;

R^4 is aryl or heteroaryl;

L^2 is C_1 - C_6 -alkyl; and

n is 10-200.

2. A Cationic-Based Lipid Encapsulation System (CaBLES) comprising:
one or more (PEG)-lipid conjugates of claim 1;
one or more non-cationic lipids, and
one or more a cationic lipids.

3. A Lipid-Based Particle, comprising:
one or more (PEG)-lipid conjugates of claim 1;
one or more non-cationic lipids, and
one or more cationic lipids, and
one or more a therapeutic agents.

4. The CaBLES of claim 2, or the Lipid-Based Particle of claim 3, wherein the PEG-lipid conjugate comprises 0.1 to about 20 weight/weight percent of total lipid in the particle.

5. The CaBLES of claim 2, or the Lipid-Based Particle of claim 3, wherein one or more non-cationic lipids is chosen from cholesterol, cholesterol sulfate, ceramide, sphingomyelin, lecithin, sphingomyelin, egg sphingomyelin, milk sphingomyelin; egg phosphatidylcholine, hydrogenated egg phosphatidylcholine, hydrogenated soybean phosphatidylethanolamine, egg phosphatidylethanolamine, hydrogenated soybean phosphatidylcholine, soybean phosphatidylcholine, 1,2-dilauroyl-sn-glycerol, 1,2-dimyristoyl-sn-glycerol, 1,2-dipalmitoyl-sn-glycerol, 1,2-distearoyl-sn-glycerol, 1,2-dilauroyl-sn-glycero-3-phosphatidic acid, 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid, 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, 1,2-distearoyl-sn-glycero-3-phosphatidic acid, 1,2-diarachidoyl-sn-glycero-3-phosphocholine, 1,2-dilauroyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine, dioleoylphosphatidylcholine, 1,2-dierucoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-stearoyl-sn-glycero-3-

phosphocholine, 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-myristoyl-2-lyso-sn-glycero-3-phosphocholine, 1-palmitoyl-2-lyso-sn-glycero-3-phosphocholine, 1-stearoyl-2-lyso-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-O-ethyl-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; 1,2-distearoyl-sn-glycero-3-phosphocholine; 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine, dioleoylphosphatidylethanolamine, palmitoyl-oleoyl-phosphatidylethanolamine, dioleoylphosphatidylglycerol, 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilauroyl-sn-glycero-3-phosphoglycerol, 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol, 1,2-dimyristoyl-sn-glycero-3-phospho-sn-1-glycerol, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol, 1,2-distearoyl-sn-glycero-3-phosphoglycerol, 1,2-distearoyl-sn-glycero-3-phospho-sn-1-glycerol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine, 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine, 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine, 1,2-distearoyl-sn-glycero-3-phospho-L-serine, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine or a mixture thereof.

6. The CaBLES of claim 2, or the Lipid-Based Particle of claim 3, wherein the non-cationic lipid comprises about 5 to about 90 weight/weight percent of total lipid in the particle.

7. The CaBLES of claim 2, or the Lipid-Based Particle of claim 3, wherein the cationic lipid is N,N-dioleoyl-N,N-dimethylammonium chloride, DC-Chol; 1,3-dioleoyloxy-2-(6-carboxyspermyl)-propyl amide, dioctadecylamidoglycyl spermine, N,N-distearyl-N,N-dimethylammonium bromide, N-(2,3-dioleoyloxy)propyl)-N,N-

dimethylammonium chloride, 1,2-dioleoyl-3-trimethylammonium-propane chloride, 1,2-dilinoyl-3-dimethylammonium-propane, N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride, 1,2-dioleoyl-3-dimethylammonium propane, 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane; didodecyldimethylammonium bromide, dioleyloxy-N-(2-sperminecarboxamido)ethyl)-N,N-dimethyl-1-propanaminiumtrifluoroacetate, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide, 1,2-dioleoylcarbaryl-3-dimethylammoniumpropane, tetramethyltetrapalmitoyl spermine, tetramethyltetraoleyl spermine, tetramethyldioleyl spermine, tetramethyltetramyristyl spermine, tetramethyltetralauryl spermine, 1-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)pyrrolidine; N,N-dimethyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(3-(1H-imidazol-1-yl)propyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; 1-methyl-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)piperazine; 4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)morpholine; N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N'-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)ethane-1,2-diamine; N-(2-(4-methylpiperazin-1-yl)ethyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(2-(1H-imidazol-4-yl)ethyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N,N-dimethyl-N-(3-(4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)piperazin-1-yl)propyl)amine; 1,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propan-2-amine; N-((1-methylpiperidin-4-yl)methyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; N-methyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-(3-pyrrolidin-1-ylpropyl)amine; N-(3-((4-

methylpiperazin-1-yl)methyl)benzyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-
 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)amine; N-methyl-N-((1-
 methylpiperidin-4-yl)methyl)-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-
 octadeca-9,12-dienyloxy)methyl)ethyl)amine; N,N,N'-trimethyl-N'-(2-((9Z,12Z)-
 octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)propane-
 1,3-diamine; N-methyl-N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-
 9,12-dienyloxy)methyl)ethyl)-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine; 1-(2-(1H-
 imidazol-1-yl)ethyl)-4-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-(((9Z,12Z)-octadeca-
 9,12-dienyloxy)methyl)ethyl)piperazine; N-(2-((9Z,12Z)-octadeca-9,12-dienyloxy)-1-
 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)ethyl)-N-((2-pyrrolidin-1-ylpyridin-3-
 yl)methyl)amine; (9Z,9'Z,12Z,12'Z)-2-(4-methylpiperazin-1-yl)propane-1,3-diyl
 dioctadeca-9,12-dienoate; (9Z,9'Z,12Z,12'Z)-2-(3-(pyrrolidin-1-yl)propylamino)propane-
 1,3-diyl dioctadeca-9,12-dienoate; 1-methyl-4-(3-((9Z,12Z)-octadeca-9,12-dienyloxy)-2-
 (((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)piperazine; 1-(3-((9Z,12Z)-octadeca-
 9,12-dienyloxy)-2-(((9Z,12Z)-octadeca-9,12-dienyloxy)methyl)propyl)pyrrolidine; N-(3-
 aminopropyl)-N'-{3-[2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-{[(9Z,12Z)-octadeca-
 9,12-dienyloxy]methyl}ethyl]amino}propyl} butane-1,4-diamine; N-(3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl)-N-(3-
 pyrrolidin-1-ylpropyl)amine; N,N-dimethyl-N-(3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-
 {[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl)amine; 3-[(9Z,12Z)-octadeca-9,12-
 dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl 2-
 (diethylamino)ethylcarbamate; 3-[(9Z,12Z)-octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-
 octadeca-9,12-dienyloxy]methyl}propyl 2-pyrrolidin-1-ylethylcarbamate; 3-[(9Z,12Z)-
 octadeca-9,12-dienyloxy]-2-{[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}propyl 2-
 (dimethylamino)ethylcarbamate; 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-{[(9Z,12Z)-
 octadeca-9,12-dienyloxy]methyl}ethyl)-4-(2-pyrrolidin-1-ylethyl)piperazine; N-(2-[(9Z)-
 octadec-9-enyloxy]-1-{[(9Z)-octadec-9-enyloxy]methyl}ethyl)-N-(3-pyrrolidin-1-
 ylpropyl)amine, 1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-{[(9Z,12Z)-octadeca-9,12-
 dienyloxy]methyl}ethyl)azetidine, 2-methyl-1-(2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-
 {[(9Z,12Z)-octadeca-9,12-dienyloxy]methyl}ethyl)aziridine, 1-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}piperidine, 4-{3,4-bis[(9Z,12Z)-octadeca-9,12-

dienyloxy]butyl}morpholine, N,N-diethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, N,N-dimethyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 phenylpiperazine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-
 methylpiperazine, N-(2-methoxyethyl)-N-methyl-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butan-1-amine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-
 methoxyphenyl)piperazine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-
 N,N',N'-trimethylethane-1,2-diamine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}-N-methyl-N-(2-pyridin-2-ylethyl)amine, N-benzyl-N-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-methylamine, N-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}-N-(4-fluorobenzyl)-N-methylamine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-(2-fluorophenyl)piperazine, N-benzyl-N-
 {3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-ethylamine, N-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}-N-ethyl-N',N'-dimethylethane-1,2-diamine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpiperidin-4-amine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N-dimethylpyrrolidin-3-amine, N,N-
 bis(2-methoxyethyl)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butan-1-amine, 1-{3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-4-methoxypiperidine, 1-{(3R)-3,4-
 bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}pyrrolidine, 1-{(3S)-3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butyl}pyrrolidine, N-{(3R)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}-N,N-diethylamine, N-{(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}-N,N-diethylamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butyl}pyrrolidine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-N,N-diethylamine, 2-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-1-methylpyrrolidine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)aziridine, 1-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-4-methylpiperazine, N-(2-{3,4-bis[(9Z,12Z)-octadeca-9,12-
 dienyloxy]butoxy}ethyl)-N,N-dimethylamine, 4-(diethylamino)-2-[(9Z,12Z)-octadeca-
 9,12-dienoyloxy]butyl (9Z,12Z)-octadeca-9,12-dienoate, 1-(2-{3,4-bis[(9Z,12Z)-
 octadeca-9,12-dienyloxy]butoxy}ethyl)pyrrolidine, N,N-diethyl-N-(2-{2-[(8Z,11Z)-
 heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-

yl}ethyl)amine, 1- {[(9Z)-octadec-9-enoyloxy]methyl}-3-pyrrolidin-1-ylpropyl (9Z)-octadec-9-enoate, 1-{3,4-bis[(9Z)-octadec-9-enyloxy]butyl}pyrrolidine, 1-{[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoyloxy]methyl}-3-pyrrolidin-1-ylpropyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate, (3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl 3-pyrrolidin-1-ylpropylcarbamate, 1-[3,4-bis(octadecyloxy)butyl]pyrrolidine, 1-[3,4-bis(hexadecyloxy)butyl]pyrrolidine, 1-{3,4-bis[(9E)-hexadec-9-enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E)-octadec-9-enyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9E,12E)-octadeca-9,12-dienyloxy]butyl}pyrrolidine, 1-{3,4-bis[(9Z,12Z,15Z)-octadeca-9,12,15-trienyloxy]butyl}pyrrolidine, N¹-(3S)-3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N³,N³-diethyl-beta-alaninamide, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-[3-(1H-imidazol-1-yl)propyl]amine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N,N',N'-trimethylpropane-1,3-diamine, 1-(1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}pyrrolidin-3-yl)-1H-imidazole, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N-(3-pyrrolidin-1-ylpropyl)amine, N-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-N',N'-dimethylpropane-1,3-diamine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}azetidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2-methylpyrrolidine, 1-{3,4-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]butyl}-2,5-dimethylpyrrolidine, are 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-1H-imidazole, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methyl-1,4-diazepane, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-phenylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-pyridin-2-ylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperidine, 4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)morpholine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-((2S)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-ethylpiperazine, N-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N-methyl-N-(3-(pyrrolidin-1-ylmethyl)benzyl)amine, N-(2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)ethyl)-N,N-dimethylamine, 1-((2S)-2,3-bis((9Z,12Z)-

octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-((2R)-2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-methylpiperazine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-4-(2-pyrrolidin-1-ylethyl)piperazine, 2-(4-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)piperazin-1-yl)pyrimidine, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)-N,N-diethylpyrrolidin-3-amine, 1-((9Z,12Z)-octadeca-9,12-dienyloxy)-3-pyrrolidin-1-ylpropan-2-ol, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 2-[(9Z,12Z)-octadeca-9,12-dienyloxy]-1-(pyrrolidin-1-ylmethyl)ethyl (9Z,12Z)-octadeca-9,12-dienoate, 1-({2-[(8Z,11Z)-heptadeca-8,11-dienyl]-2-[(9Z,12Z)-octadeca-9,12-dienyl]-1,3-dioxolan-4-yl} methyl)pyrrolidine, 1-{2,3-bis[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]propyl}pyrrolidine, 1-{3-[(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenyloxy]-2-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9E,12E)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-{2-[(9E,12E)-octadeca-9,12-dienyloxy]-3-[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidine, 1-[2,3-bis(tetradecyloxy)propyl]pyrrolidine, 1-[2,3-bis(octadecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-[2,3-bis(dodecyloxy)propyl]pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}pyrrolidin-3-ol, 1-{3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-[(9Z)-octadec-9-enyloxy]propyl}pyrrolidine, 1-{2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy]propyl}-N,N-dimethylpyrrolidin-3-amine and 1-[3-[(9Z,12Z)-hexadeca-9,12-dienyloxy]-2-(tetradecyloxy)propyl]pyrrolidine, or a mixture thereof.

8. The CaBLES of claim 2, or the Lipid-Based Particle of claim 3, wherein the cationic lipid comprises about 2 to about 60 weight/weight percent of total lipid in the particle.

9. The compound of claim 1 which is 2-(tetradecyloxy)-1-
((tetradecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate,
2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate,
2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate,
2-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
hexatetracontaoxaooctatriacontahectanamidopropane-1,3-diyl ditetradecanoate,
2-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
hexatetracontaoxaooctatriacontahectanamidopropane-1,3-diyl dipalmitate,
2-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,94,97,100,103,106,109,112,115,118,121,124,127,130,133,136-
hexatetracontaoxaooctatriacontahectanamidopropane-1,3-diyl distearate,
N-(2-(hexadecyloxy)-1-((hexadecyloxy)methyl)ethyl)-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
hexatetracontaoxanonatriacontahectan-139-amide
N-(2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl)-
2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,

92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
 hexatetracontaoxanonatriacontahectan-139-amide, or
 N-(2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl)-
 2,5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86,89,
 92,95,98,101,104,107,110,113,116,119,122,125,128,131,134,137-
 hexatetracontaoxanonatriacontahectan-139-amide.

10. The Lipid-Based Particle of claim 3, wherein the therapeutic agent is RNA, antisense oligonucleotide, a DNA, a plasmid, a ribozymal RNA (rRNA), a micro RNA (miRNA), transfer RNA (tRNA), a small inhibitory RNA (siRNA), small nuclear RNA (snRNA), an antigen, fragments thereof, a protein, a peptide, a small-molecule, or a mixture thereof.

11. The Lipid-Based Particle of claim 3, wherein said PEG lipid conjugate is about 0.1-20 weight/weight % of total lipid in particle, DSPC is about 1-30 weight/weight % of total lipid in particle, cholesterol is about 5-45 weight/weight % of total lipid in particle, and said cationic lipid is about 5-60 weight/weight % of total lipid in particle.

12. A pharmaceutical composition comprising a Lipid-Based Particle of claim 3 and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition of claim 12, wherein said Lipid-Based Particle comprises cholesterol, DSPC, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,
 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
 hexatetracontaoxanonatriacontahect-1-ylcarbamate and one or more nucleic acids.

14. A pharmaceutical composition of claim 13, wherein said 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl

3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate 1 is about 1-25 weight/weight % of total lipid in particle, said DSPC is about 1-30 weight/weight % of total lipid in particle, said cholesterol is about 5-45 weight/weight % of total lipid in particle, and 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

15. The Lipid-Based Particle of claim 3, wherein said non-cationic lipids are cholesterol and DSPC, said cationic lipid is 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, said PEG-lipid conjugate is 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate, and said therapeutic agent is siRNA.

16. The Lipid-Based Particle of claim 15, wherein said 2-(tetradecyloxy)-1-((tetradecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-
hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, said DSPC is about 1-30 weight/weight % of total lipid in particle, said cholesterol is about 5-45 weight/weight % of total lipid in particle, and said 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

17. A pharmaceutical composition of claim 12, wherein said Lipid-Based Particle comprises, cholesterol, DSPC, 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl
3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,

93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate and one or more nucleic acids.

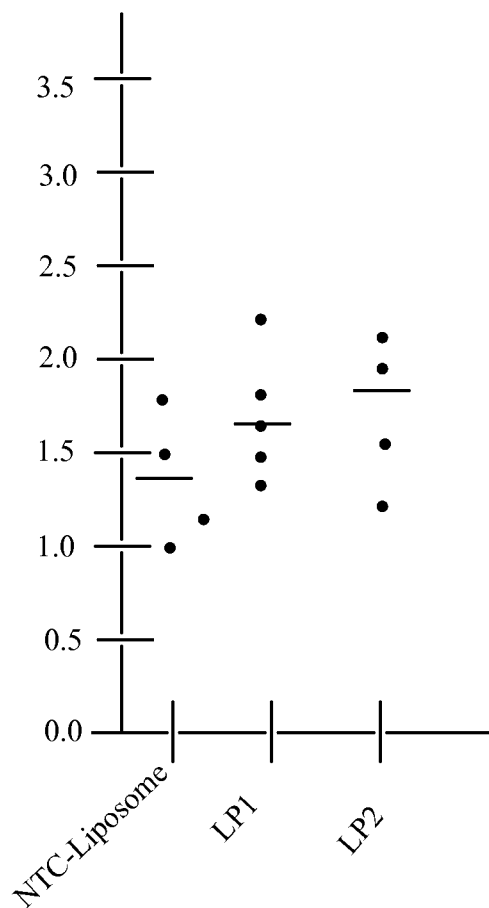
18. A pharmaceutical composition of claim 17, wherein said 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, said DSPC is about 1-30 weight/weight % of total lipid in particle, said cholesterol is about 5-45 weight/weight % of total lipid in particle, and 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

19. The Lipid-Based Particle of claim 3, wherein said non-cationic lipids are cholesterol and DSPC, said cationic lipid is 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine, said PEG-lipid conjugate is 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate, and said therapeutic agent is siRNA.

20. The Lipid-Based Particle of claim 19, wherein said 2-(octadecyloxy)-1-((octadecyloxy)methyl)ethyl 3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90, 93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,138-hexatetracontaoxanonatriacontahect-1-ylcarbamate is about 1-25 weight/weight % of total lipid in particle, said DSPC is about 1-30 weight/weight % of total lipid in particle, said cholesterol is about 5-45 weight/weight % of total lipid in particle, and 1-(2,3-bis((9Z,12Z)-octadeca-9,12-dienyloxy)propyl)pyrrolidine is about 5-60 weight/weight % of total lipid in particle.

21. A method of making the Lipid-Based Particle of claim 3, comprising:
- (a) mixing the cationic lipid(s), the non-cationic lipid(s) and the PEG-lipid conjugate(s);
 - (b) adding the mixture of step (a) to one or more therapeutic agents; and
 - (c) separating and purifying resulting suspension of step (b).
22. The method of claim 21, wherein said mixture of step (a) and one or more said therapeutic agents are warmed to about 60° C prior to the addition of said mixture of step (a) to one or more therapeutic agents via needle injection.
23. The CaBLES of claim 2 which effectively encapsulate therapeutic agents, with efficiencies from about 50-100%.
24. The CaBLES of claim 2 which effectively encapsulate therapeutic agents, with efficiencies from about 80-100%.
25. The Lipid-Based Particle of claim 3, wherein the ratio of one or more (PEG)-lipid conjugates, one or more non-cationic lipids, and one or more cationic lipids of claim 1, to one or more therapeutic agents is between about 50:1 to about 5:1.
26. The Lipid-Based Particle of claim 3, wherein the ratio of one or more (PEG)-lipid conjugates, one or more non-cationic lipids, and one or more cationic lipids of claim 1, to one or more therapeutic agents is between about 30:1 to about 10:1.

FIGURE 1 illustrates in vivo activity of Lipid-Based Particle 1(LP1) and Lipid-Based Particle 2 (LP2) versus a non-targeted composition (NTC).



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/056446

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/00 A61K47/10 A61K47/14 A61K9/127

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)

A61K A61P

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 practical, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/019486 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; MACLACHLAN IAN [CA]; ROBBINS MARJORI) 21 February 2008 (2008-02-21) page 9 - page 34; claims 1-40 paragraph [0149] - paragraph [0242]	1-26
X	WO 2006/074546 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; MACLACHLAN IAN [CA]; AMBEGIA ELLEN G) 20 July 2006 (2006-07-20) page 1, paragraph 14 - page 184, paragraph 281; claims 1-55	1-26
X	WO 2005/007196 A2 (PROTIVA BIOTHERAPEUTICS INC [CA]; MACLACHLAN IAN [CA]; AMBEGIA ELLEN G) 27 January 2005 (2005-01-27) page 6 - page 12 page 20 - page 27; claims 1-87 page 60 - page 73; examples 7-9	1-26
	-/-	

☒ 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 document 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
22 December 2009	30/12/2009
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Kling, Isabelle

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/056446

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

☒

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☐

on paper

☒

in electronic form

c. time of filing/furnishing

☒

contained in the international application as filed

☐

filed together with the international application in electronic form

☐

furnished subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/056446

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/121348 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; MACLACHLAN IAN [CA]; PALMER LORNE R) 22 December 2005 (2005-12-22) page 7 - page 13 page 18 - page 26 page 36 - page 45 page 53 - page 64; claims 1-34; examples 9-18 -----	1-26
X	WO 2005/026372 A1 (PROTIVA BIOTHERAPEUTICS INC [CA]; HEYES JAMES [CA]; MACLACHLAN IAN [CA]) 24 March 2005 (2005-03-24) page 2 - page 21 page 28 - page 42; claims 1-62; examples 5-10 -----	1-26
X	BEILSTEIN,, 1 January 2005 (2005-01-01), XP002535770 the whole document -----	1
E	WO 2009/129395 A1 (ABBOTT LAB [US]; DANDE PRASAD A [US]; HANSEN TODD M [US]; HUBBARD ROBE) 22 October 2009 (2009-10-22) page 4; claims 1-81 -----	1-26
E	WO 2009/129385 A1 (ABBOTT LAB [US]; DANDE PRASAD A [US]; HANSEN TODD M [US]; HUBBARD ROBE) 22 October 2009 (2009-10-22) claims 1-33 -----	1-26
E	WO 2009/129387 A2 (ABBOTT LAB [US]; DANDE PRASAD A [US]; HANSEN TODD M [US]; HUBBARD ROBE) 22 October 2009 (2009-10-22) claims 1-45 -----	1-26
E	WO 2009/129319 A2 (PROTIVA BIOTHERAPEUTICS INC [CA]; US GOVERNMENT [US]; MACLACHLAN IAN [CA]) 22 October 2009 (2009-10-22) page 33, paragraph 232 - page 39, paragraph 257; claims 35-37 -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/056446

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008019486 A1	21-02-2008	AU 2007284036 A1 CA 2660204 A1 EP 2056880 A1 US 2008171716 A1	21-02-2008 21-02-2008 13-05-2009 17-07-2008
WO 2006074546 A1	20-07-2006	NONE	
WO 2005007196 A2	27-01-2005	AU 2004257373 A1 CA 2532228 A1 EP 1648519 A2 JP 2007528863 T KR 20060028655 A	27-01-2005 27-01-2005 26-04-2006 18-10-2007 30-03-2006
WO 2005121348 A1	22-12-2005	AU 2005252273 A1 CA 2569664 A1 EP 1766035 A1 JP 2008501730 T	22-12-2005 22-12-2005 28-03-2007 24-01-2008
WO 2005026372 A1	24-03-2005	AU 2004272646 A1 CA 2551022 A1 CN 1882693 A EP 1664316 A1 JP 2007505954 T KR 20060120009 A	24-03-2005 24-03-2005 20-12-2006 07-06-2006 15-03-2007 24-11-2006
WO 2009129395 A1	22-10-2009	NONE	
WO 2009129385 A1	22-10-2009	NONE	
WO 2009129387 A2	22-10-2009	NONE	
WO 2009129319 A2	22-10-2009	NONE	